Barriers to bacterial motility on unsaturated surfaces

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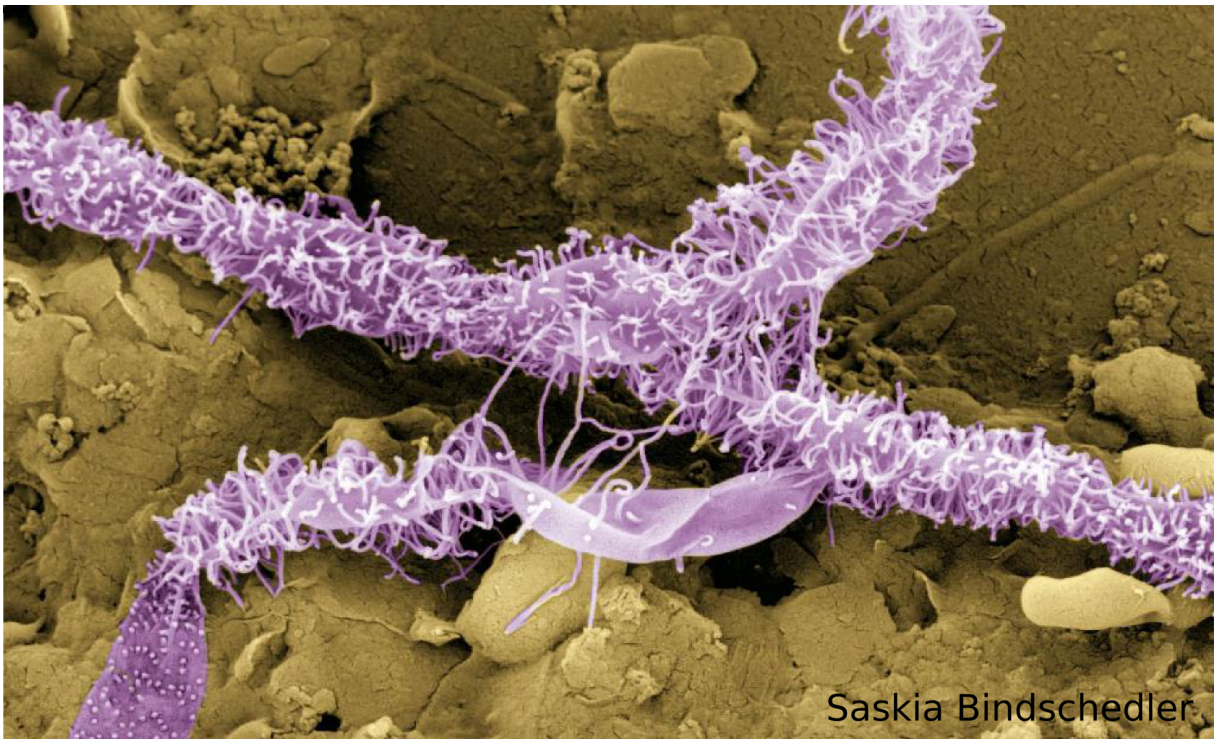

SME 2013

5th Swiss Microbial Ecology Meeting, Centre Loewenberg, Murten Switzerland

4.-6. February 2013

Contents

Welcome

Dear participants of the SME-2013 meeting,

We are pleased to welcome you to the Swiss Microbial Ecology (SME) Meeting, which is the number five in a series of meetings, established to bring together Swiss microbial ecologists from all parts of the country and all areas of microbial ecology.

After the first four stimulating meetings in Neuchâtel (2004), Bellinzona (2006), Einsiedeln (2009), and Engelberg (2011), it is time to meet again and to exchange exciting findings and important information on how microorganisms interact with each other and with their environments. The meeting, organized by the laboratory of microbiology from the University of Neuchâtel with the help of scientist from other Swiss institutions, has attracted once more close to 80 participants with a total of 30 oral and 27 poster presentations. In addition, three keynote presentations complete a diverse and stimulating scientific program. We wish to thank all of the participants in advance for their valuable contribution that makes possible this meeting. Furthermore we are very grateful to our sponsors that allow us to maintain the registration fees for the SME meeting affordable for participants from all stages in the academic and professional careers. The SME meeting is a "melting pot" in which senior scientist and young researchers can exchange results and ideas, and all this is possible thanks to the support of those sponsoring the meeting. Finally, we would like also to thank the Conférence Universitaire de Suisse Occidentale (CUSO) for its support in the organization of two parallel satellite symposia in Metagenomics and Soil Logistics, which are an opportunity for PhD students to enrich their education.

In a field that evolves as fast as Microbial Ecology does it is difficult to imagine an specific unifying theme. Therefore, the organizing committee for SME-2013 has put a lot of emphasis in covering different aspects of the study of Microbial

Ecology and to include people working in all microbial groups (bacteria, fungi and protist). However, for this meeting, two topics obtained a particularly significant response: "Microbial Interactions" and "Microbial Communities". Both topics emphasized the importance of the realization that in ecology an organism is not an isolated entity, but rather the result of its interaction with other organisms and their environment. Most microorganisms are still barely known and their functions and interactions in the environment are still obscure. The diversity and complexity of microbes and their interactions make the research in this topic very exciting and relevant.

We are looking forward to a stimulating conference and hope that you will all profit from your participation in the SME-2013 meeting. We are convinced that this meeting, with its scientific presentations, fruitful discussions and the many opportunities for establishing new contacts and collaborations will contribute to the advance of Microbial Ecology in Switzerland in the future.

With best wishes,

The organizing committee

Pilar Junier, Daniel Job, Enrique Lara, Franco Widmer, Christof Holliger, Julien Maillard, Pierre Rossi, Jakob Zopfi, Dave Johnson, Thomas Egli

Organization

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Program

Monday, 4th February 2013

Time	Activity
12:00	Registration and provision of all power points and poster presentations
15:30	Welcome coffee/tea break
16:00 - 16:15	Welcome and general information (Pilar Junier)
16:15 - 17:15	**Special lecture: Thomas Egli** Mixed substrate growth and microbial competition
17:30 - 18:30	Poster session: Presentation posters 1-12
18:30 - 19:30	Welcome apero
19:30	Dinner

Tuesday, 5th February 2013

Session Omics (chairman Pilar Junier)

08:30 - 09:15	**Key lecture: Greg Caporaso** Ultra-high-throughput microbial ecology: software, sequencing and practice for studying tens of thousands of environments
09:15 - 09:30	**Martin Hartmann** Soil Compaction Caused by Logging Operations Persistently Alters Microbial Diversity, Structure and Function
09:30 - 09:45	**Fabienne Wichmann** Dairy cow manure harbors and unexpected divergence and diversity of antibiotic resistance genes
09:45 - 10:30	Coffee and Tea break and Poster visit

Session Microbial interactions I (chairman David Johnson)

10:30 - 11:00	**Rolf Kuemmerli** Competitive and cooperative interactions among siderophore-producing and non-producing strains in Pseudomonas aeruginosa
11:00 - 11:15	**Felix Goldschmidt** Mutualistic interactions maintain diversity in expanding microbial communities
11:15 - 11:30	**Laure Weisskopf** A smelly world: how bacterial volatiles influence the growth of plants and of phytopathogenic fungi
11:30 - 11:45	**Marie Marchal** The evolution and stabilization of mutualistic interactions in microbial ecosystems
11:45 - 12:00	**Anaele Simon** Abundance, diversity and activity of fungal highways in natural ecosystems - a new approach
12:00 - 13:30	Lunch Break

Tuesday, 5th February 2013 - continuation

Session Protist Ecology and microbial interactions II (Chairman Enrique Lara)

13:30 - 13:45	**Enrique Lara** Niche-driven and geographically influenced patterns of diversity characterise euglyphid testate amoebae
13:45 - 14:00	**Anush Kosakyan** Estimation of the cloning biases in the evaluation of diversity in microbial eukaryotes: the case of the Nebela tinctabohemica-collaris complex
14:00 - 14:15	**Christophe Seppey** Euglyphida (Cercozoa; Rhizaria; Eukaryota) communities under pig cadavers by high throughput sequencing
14:15 - 14:30	**Saskia Bindschedler** Microbial interactions in the oxalate-carbonate pathway: fungal networks promote the activity of oxalotrophic bacteria
14:30 - 14:45	**Sebastian Dirren** The amoeba Nuclearia sp. from Lake Zurich live in concert with ecto- and endosymbiotic bacteria

14:45 - 15:15 Coffee Break

Tuesday, 5th February 2013 - continuation

Session Microbial Communities I (Chairman Pierre Rossi)

15:15 - 15:30	**David Gregory Weissbrodt** PyroTRF-ID: a novel bioinformatics methodology for the affiliation of terminal-restriction fragments using 16S rRNA gene pyrosequencing data
15:30 - 15:45	**Michel Aragno** ORION (ORganic waste management by a small-scale innovative automated system of anaerobic digestION): a FP7 project on *in situ* biomethanization of specific wastes
15:45 - 16:00	**Daniel Bravo** Identification of active oxalotrophic bacteria by BrdU labeled-DNA and their importance in the oxalate-carbonate pathway in natural environments
16:00 - 16:15	**Sevasti Filippidou** Microbial communities in geothermal sites. Are endospore-forming bacteria favored?
16:15 - 16:30	**Nejc Stopnisek** Biogeography of soil Burkholderia populations
16:30 - 18:00	Coffee Break and poster session posters 13-27
19:00	Social event

Wednesday, 6th February 2013

Session Mycology (chairman Daniel Job)

08:30 - 09:15	**Key lecture: Arnaud Deschene** Barriers to bacterial motility on unsaturated surfaces
09:15 - 09:30	**Mireille Dessimoz** Alternative methods to control Verticillium dahliae: efficacy and impact on non-target soil fungi
09:30 - 09:45	**Pilar Junier** Fungal control of dispersion and activity of bacteria in unsaturated environments
09:45 - 10:00	**Edith Joseph** Biopatinas or the use of fungi for the conservation-restoration of copper-based artefacts
10:00 - 10:30	Coffee and Tea break

Wednesday, 6th February 2013 - continuation

Session Microbial Communities II (chairman Julien Maillard)

10:30 - 10:45 **David Johnson**
Is microbial diversity important for the functional performance of wastewater treatment plant microbial communities ?

10:45 - 11:00 **Alexandra Dostal**
Dietary iron supplementation of human gut microbiota associated rats impacts gut microbiota composition and metabolic activity

11:00 - 11:15 **Matthieu Bueche**
New molecular method for the quantification of endospore-forming bacteria

11:15 - 11:30 **Hannes Gamper**
Experimental community assembly of arbuscular mycorrhizal fungi in the field - SMRT ccs of tag-labeled multiplexed PCR amplicons

11:30 - 11:45 **Thomas Rime**
Pyrosequencing based assessment of microbial communities in ice sediments and various depths along the Damma soil chronosequence

11:45 - 12:00 **Sabine Tanner**
Validation of a novel in vitro fermentation model, PolyFermS®, for the swine proximal colon

12:00 - 13:30 Lunch Break

Wednesday, 6th February 2013 - continuation

Session Biogeochemical cycling (Chairman Jakob Zopfi)

13:30 - 13:45	**Lea Steinie** Microbial methane oxidation in the Arctic Ocean offshore Svalbard
13:45 - 14:00	**Jen-How Huang** Characterizing iron and arsenate reduction of arsenate-reacted ferrihdyrite by Shewanella putrefaciens strain CN-32
14:00 - 14:15	**Tina Wunderlin** Paleolimnology of Lake Geneva using endospore-forming bacteria
14:15 - 14:30	**Helmut Burgmann** Antibiotic resistance as an emerging environmental contaminant
14:30 - 15:00	Concluding remarks

Poster Sessions

Presentation posters 1-12 (Monday, 4th, 17:30 - 18:30)

P1	**Aamani Rupakula**: Corrinoid auxotrophy in the obligate organohalide respiring Dehalobacter restrictus
P2	**Pascale Flury**: How can plant-associated pseudomonads with antifungal activity become insect pathogens?
P3	**Lukas Hunziker**: Bacteria as biocontrol agents of phytopathogenic fungi: the role of volatile organic compounds
P4	**Stephen Mackay**: Pelletization of Micro-algae by Induced Lichen Formation through Co-culture with Filamentous Fungi
P5	**Veronica Bergottini**: Testing plant growth promoting rhizobacteria (PGPR) isolates as inoculants for Ilex paraguariensis (yerba mate)
P6	**Michael P. Baumgartner**: Predator-prey interactions induce rapid adaptation in a freshwater bacterial isolate
P7	**Anita Zumsteg**: Identification of Biomass Utilizing Bacteria in a Carbon Depleted Glacier Forefield Soil by the Use of 13C-DNA-Stable Isotope Probing
P8	**Martin Hartmann**: The Influence of Agricultural Management Practices on the Soil Microbiome as Revealed by Massively Parallel Pyrosequencing
P9	**Carlotta Fabbri**: Degradation of raffinose by a novel strain of Pseudomonas
P10	**Andy Lutz**: Genetic tools for genotyping, detection, and quantification of Metharizium species in soil
P11	**Ludovic Roussel-Delif**: New methods to quickly screen diversity of endospore-forming bacteria in environmental samples
P12	**Hannes Gamper**: Experimental confrontation of natural arbuscular mycorrhizal fungal assemblages in the field - Study of community assembly

Presentation posters 13-27 (Tuesday, 5th, 16:30 - 18:00)

P13 **Cindy Kunze**: Characterization of the metallo-cofactors of the tetrachloroethene reductive dehalogenase purified from Sulfurospirillum multivorans

P14 **Géraldine Buttet**: Functional genotyping of Sulfurospirillum spp. in mixed cultures allowed the identification of a new PCE reductive dehalogenase

P15 **Yuhui Xu**: The potential application of Amanita muscaria in vanadium bioremediation

P16 **Sathiyanarayanan Ganesan**: Removal of Copper (Cu) from Industrial waste water using Bacillus spp.: An economical and viable approach

P17 **Alejandro Gómez Mejía**: Implementation of a genetic transformation strategy to improve the biological CO2 capture in Chlorella sp. and Scenedesmus sp.

P18 **Martina Praveckova**: Metagenomic analyses of PCB-degrading consortia present in sediment-free microcosms revealed novel microbial structures

P19 **Zoe Bont**: Antifungal susceptibility testing based on the bioluminescence by Armillaria cepistipes, formely unknown to produce light

P20 **Francesca Dennert**: Abundance and genotype diversity of Metarhizium spp. in a grassland in northern Switzerland

P21 **Isabelle Pfändler**: Are microsatellite analysis and elongation factor 1α sequencing useful tools for discriminating exotic from native Metarhizium strains?

P22 **Nicole Bichsel**: Is the entomopathogenic fungus Beauveria brongniartii also an endophyte?

P23 **Amandine Pillonel**: Bacterial Spores Involved in Mineral Oxidation and Precipitation

P24 **Emiliano Stopelli**: Biological ice nucleation at tropospheric cloud heights

P25 **Jakob Zopfi**: Microbial community structures and biogeochemistry of pillow-like sediment structures in Lake Geneva

P26 **José Santos Cáceres**: Elimination of antibiotic resistance genes by an ultrafiltration pilot plant at the Waste Water Treatment Plant Lausanne, Switzerland

P27 **Rainer Follador**: Microsynth's 16S Metagenomic analysis and Transcriptomic analysis

Abstracts

Keynote presentations

Mixed substrate growth and microbial competition

Thomas Egli[1]

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Availability of carbon/energy sources and temperature are the two environmental factors that severely restrict heterotrophic growth in most ecosystems. TOC concentrations in ground, drinking and surface waters are typically in the range of 0.5-5 mg/L, but most of this is present in a polymeric, inaccessible form for microbes. Concentrations of available carbon compounds (so-called assimilable organic carbon, AOC) are usually in the range of 10-100 µg/L, those of individual sugars or amino acids are not higher than a few µg/L. Until recently microbiologists assumed that such nutrient-poor (oligotrophic) environments are "deserts" for life, and that the majority of bacterial cells seen in the microscope are dead, dormant or at least severely starved. Nevertheless, bacterial cell numbers recorded in these environments typically are in the range of 10^5 - 10^6 per mL. Over the last years we have learnt that most of these microbes are perfectly alive, metabolizing and ready to grow when given the chance. Hence, microbes have adapted and developed strategies to cope with this situation.

Laboratory studies with pure cultures suggest that bacterial cells have developed two strategies to live under such conditions. The first strategy is to perform a "multivorous" way of life by taking up and metabolizing dozens of different carbon substrates simultaneously (i.e., they are NOT specializing on a particular substrate, which they can take up with very high affinity). This "mixed substrate growth" equips the cell with a kinetic advantage and metabolic flexibility. Simultaneous utilization of a multitude of carbon substrates allows fast growth at minute concentrations of individual substrates. The second strategy is to minimize maintenance requirements (unfortunately we still know little about how this is achieved).

Recently, flow cytometry has been employed to study microbial growth in very dilute, nutrient-poor environments. The technique allows fast and easy quantification of microbial growth of natural bacterial communities, including "uncultivable" members, under environmental conditions. It also allows investigating microbial growth, survival and competition in aquatic environments, e.g., when combined with strain-specific fluorescent immunoprobes, growth and competition of pathogens with the indigenous microbial flora.

Although the basics seem established, there is much missing, particularly concerning concepts of competition kinetics in complex environments, including transient and oscillating conditions. The concepts presently used are mostly those developed some 50 years ago by the pioneers in microbial competition. I will address some of most important issues, existing gaps, and will try to demonstrate in a few examples of competition experiments under apparently simple (mutant selection in "pure" chemostat cultures) and more complex conditions (competition of pathogens with natural bacterial freshwater flora; competition of Low Nucleic Acid (LNA) and High Nucleic Acid (HNA) bacterial clusters in in-house drinking water installations) in what direction the field might move.

Ultra-high-throughput microbial ecology: software, sequencing and practice for studying tens of thousands of environments

Greg Caporaso[1,2]

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[2]Assistant Professor, Institute for Genomics and Systems Biology, Argonne National Laboratory, Argonne, IL 60439, USA, gregcaporaso@gmail.com

Microbial ecology is an exciting and rapidly growing area of biology, with almost weekly publications in Science, Nature, PNAS, and even popular literature sources such as The New York Times. This field also exemplifies the increasingly data-intensive nature of modern Biology: a single study can easily generate greater than 80 gigabytes of raw sequence data and is therefore multidisciplinary by requirement. In this talk I will present my recent work on increasing the scale on which microbial ecology is possible, both in terms of breadth (the types of communities that can be profiled in high-throughput) and depth (the amount of data that can be collected and analyzed). I will talk about my work on the QIIME (Quantitative Insights Into Microbial Ecology; www.qiime.org) software package, developing a community sequencing protocol for the Illumina sequencing technologies, and adopting standards to increase reproducibility and support meta-analyses in comparative genomics generally. These tools have made it possible to increase the scale of these studies by about 2000x in just two years without increasing the cost. I will conclude by presenting several projects that illustrate what is possible in ultra-high-throughput microbial ecology: for example, a timeseries analysis of the human microbiome profiling four body sites from over 100 college students with weekly sampling for ten weeks, and preliminary results from the Earth Microbiome Project.

Barriers to bacterial motility on unsaturated surfaces

Arnaud Deschene[1], Barth F. Smets[1]

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Our knowledge of the spatial organization and spatial dynamics of microbial populations in soil at a scale close to that of the microorganisms is scarce. While passive dispersal via water flow or soil biota is probably a major dispersal route, it is reasonable to consider that active dispersal also contributes to microbial spatial dynamics. In bacteria, active dispersal is enabled by a diversity of appendages and, in the case of swarming motility, by the secretion of surface active biomolecules. It is however unclear to which degree different types of motility can take place in the soil pores, a habitat characterized by complex 3D geometry and variable hydration.
To approach these questions we take advantage of the Porous Surface Model (PSM) a unique experimental platform that allows direct monitoring of microbial motion under precisely controlled matric potential. Using gfp-tagged *Pseudomonas* strains and their isogenic mutants unable to express various type of motility we aimed to quantify the physical limits of bacterial motility.
Our results demonstrate how hydration controls bacterial motility under unsaturated conditions. They can form the base of improved biodegradation models that include microbial dispersal processes.

Oral presentations

Soil Compaction Caused by Logging Operations Persistently Alters Microbial Diversity, Structure and Function

Martin Hartmann[1,2], Stefan Schmutz[1], Franco Widmer[2], Beat Frey[1]

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Soil compaction has been recognized as a major disturbance associated with logging operations, but we lack fundamental knowledge how this affects the soil microbiome. We assessed resistance and resilience of the soil microbiome after compaction and correlated our findings with changes in soil functions. Logging traffic across a soil moisture gradient installed at two different forest sites generated replicated skid trails of different impacts. Soil physical properties and fluxes of greenhouse gases were measured to assess alterations in soil functioning in these skid trails. Metagenomic DNA was extracted from soil samples collected at various time points after compaction in order to assess microbial diversity and community structure using massively parallel pyrosequencing of bacterial and fungal ribosomal markers.

The analysis of about 900,000 pyrotags revealed that compaction significantly altered diversity and structure of both bacteria and fungi. The strongest effects were observed in severely compacted soils where air and water conductivities dropped below 10% of the initial value. Sandy soils revealed higher resistance to compaction than clayey soils. Effects were most pronounced in the medium-term (180-365 days) and were less strong in the short- and long-term (30 days or 4 years), but communities in the severely compacted soils did not yet show resilience after 4 years. Taxa-treatment association analysis revealed that anaerobically respiring bacteria (e.g. sulfate- and metal-reducers) from the Firmicutes, Delta- and Betaproteobacteria as well as fungal saprobes from the Ascomycota were increased in compacted soils. Conversely, aerobically respiring bacteria from the Actinobacteria, Alpha- and Gammaproteobacteria as well as mycorrhizal fungi from the Basidiomycota were negatively affected by compaction. Accordingly, greenhouse gas fluxes significantly changed in the compacted soils, resulting in reduced carbon dioxide and increased methane and nitrous oxide emissions.

This study demonstrates that physical soil disturbance during logging alters soil functioning and that the response of the microbiome is massive and tightly linked to these changes. Taxa indicative of these conditions can now help to monitor resistance and resilience of various soil types after logging operations.

Dairy cow manure harbors and unexpected divergence and diversity of antibiotic resistance genes

Fabienne Wichmann[1], Nikolina Udikovic Kolic[1], Sheila Andrew[2], Jo Handelsman[1]

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The prevalence of antibiotic resistance is rapidly increasing, and thus represents a major threat to public health. Massive use of antibiotics in animal agriculture is thought to be a main source of novel antibiotic resistance determinants. Further, the application of manure as fertilizers facilitates the dissemination of these antibiotic resistant bacteria and their underlying resistance genes into the environment. However, our knowledge of the identity and diversity of antibiotic resistance determinants in manure is still limited. To gain insight into the resistome of dairy cow manure, we constructed ten metagenomic fosmid and small-insert libraries originating from manure sampled from four different cows. The metagenomic libraries covered in total 27.2 Gb of DNA, which we screened for functional antibiotic resistance genes to different classes of antibiotics including β-lactams, cephalosporins, phenicols, aminoglycosides and tetracycline. Our functional screen identified in total 70 different antibiotic resistance genes. The predicted proteins encoded by these genes were on average only 50-60% similar to protein sequences deposited in public databases. Besides different extended spectrum beta-lactamases, a great divergence of sequences within and between manure samples of the different animals was observed. This was particularly true for N-acetyltransferases and chloramphenicol-acetyltransfereses conferring resistance to kanamycin and chloramphenicol, respectively. Overall, our study demonstrates that manure represents a highly diverse reservoir of antibiotic resistance genes, and therefore significantly extends the current knowledge of functional resistance genes encoded by bacteria in animal gut microbiomes. In the future, it will provide an indispensible resource for antibiotic resistance management strategies.

Competitive and cooperative interactions among siderophore-producing and non-producing strains in *Pseudomonas aeruginosa*

Rolf Kuemmerli[1]

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The secretion of iron-chelating siderophores is essential for bacteria to cope with the ubiquitous iron limitation in nature and to establish infections within hosts. Siderophore production can be understood as a cooperative behavior that is costly for the individual cell, but provides benefits to other cells in the vicinity. Explaining such cooperation is challenging because the spread of non-cooperative mutants that exploit and displace cooperative individuals is expected. Here, we address this challenge by investigating ecological factors that influence selection for or against cooperation. We do this by studying competitive interactions among siderophore-producing and non-producing strains in the opportunistic human pathogen *Pseudomonas aeruginosa*. We found that media viscosity (determining who interacts with whom), nutrient supply and chemical properties of siderophores (determining the cost and benefit of siderophore production) all significantly influence selection for cooperation. Furthermore, we found evidence for antagonistic co-evolution between producers and non-producers during experimental evolution: while producers evolved towards becoming less exploitable, non-producers evolved towards becoming more efficient exploiters. Altogether, our findings highlight the complexity of interactions among bacteria even in a simple ecosystem consisting of only two strains.

Mutualistic interactions maintain diversity in expanding microbial communities

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Microbial communities impact the biological and chemical processes occurring in nearly every habitat on earth. These communities are often extremely diverse, with some estimated to contain thousands to millions of different taxa. It is still not clear by which mechanisms this diversity evolves and can be maintained. Range expansions are common events in the history of most species and are thought to decrease microbial diversity. In this study we developed a system of mutualistically interacting bacteria to test the influence of these interactions on diversity during range expansions.

Our model system consists of two auxotrophic strains of *Escherichia coli* that are mutualistically dependent on each other. One strain cannot synthesize proline and the other tryptophan. When they are grown on minimal medium that does not contain amino acids, they can only grow if they are close to their counterpart that secretes amino acids. By adding or omitting amino acids in the growth medium we can adjust the interactions between the strains. We inoculated mixtures of these strains together in the center of agar plates and let them expand. We then measured the emerging patterns of interacting and non-interacting strains with confocal laser scanning microscopy.

We have preliminary evidence that mutualistic interactions between the two strains maintain diversity in expanding populations. When they do not interact, large sectors are forming in the expansion zone that consist of only one type of the strains. This means that a large proportion of the initial diversity is lost and corresponds with previous findings. However, when they mutualistically interact, much narrower sectors are forming so that more sectors exist in the same area. This indicates that more of the initial diversity is maintained than in the non-interacting case.

Our results demonstrate that mutualistic interactions between different cell types can maintain diversity in expanding microbial populations. Future work will investigate how co-evolution can lead to diversification in these populations.

A smelly world: how bacterial volatiles influence the growth of plants and of phytopathogenic fungi

Aurélien Bailly[2], Rita Baumgartner[2], Ulrike Groenhagen[3], Stefan Schulz[3], Leo Eberl[2], Laure Weisskopf[1]

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Increasing evidence indicates that bacteria can interact with other organisms through the emission of volatile compounds. We have recently demonstrated that plant growth promotion by bacterial volatiles is a general feature of root-associated bacteria, and especially of *Burkholderia* species. In addition to direct plant growth promotion, bacterial volatiles have been shown to inhibit the growth of phytopathogenic fungi of agronomical relevance such as *B. cinerea*, *R. solani* or *A. alternata*. Moreover, we have recently extended our investigations to volatile-mediated bacteria-bacteria interactions and observed induction of antibiotic tolerance in *Escherichia coli* when exposed to the volatiles of various *Burkholderia* strains. We are currently analysing which active molecules are responsible for these strong effects of bacterial volatiles on plants, fungi and bacteria.

The evolution and stabilization of mutualistic interactions in microbial ecosystems

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Interactions between microbial strains shape the assembly and functioning of nearly every microbial community. One important type of interaction is a metabolite-based mutualism. In this type of interaction, each strain secretes a different metabolite that is essential for the growth of other strains. The production and secretion of metabolites is often metabolically costly. If excreting metabolites is costly, how do metabolite-based mutualistic interactions evolve in the natural environment? There is empirical evidence that spatial structure can stabilize existing mutualistic interactions. Indeed, in unstructured environments, the benefits of metabolite secretion are equally accessible to all individuals. In spatially structured environments, the benefits of metabolite secretion are directed back to the individuals that provide the metabolite secretion. However there is little empirical evidence that spatial structure is an absolute precondition for the evolution of increased metabolite secretion and enhanced mutualistic interactions. To address this, we constructed an obligate metabolite-based mutualistic consortium between two auxotrophic strains of *E. coli*. Both strains can grow together in minimal medium but neither strain can grow alone. Interestingly, we observed that this mutualistic consortium created its own spatial structure when cultivated in an unstructured environment, and thus created the conditions that are theoretically conducive for metabolite secretion. This discovery led us to formulate the following hypothesis about the evolution of mutualistic interactions between microorganisms: Weak mutualistic interactions promote the evolution of spatial structure through cell aggregation, which then sets the stage for the evolution of strong mutualistic interactions. This principle could be a general mechanism that promotes the evolution of mutualistic interactions and, in turn, controls the assembly and functioning of natural microbial communities.

Abundance, diversity and activity of fungal highways in natural ecosystems - a new approach

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The so called "fungal highway" is a phenomenon in which bacteria can disperse over the fungal mycelium network, increasing bacterial activity, especially in unsaturated porous media like soils, where bacterial dispersion is usually very limited. Recent studies highlight great possibilities linked to the fungal highways, such as a better biodegradation of soil pollutants or higher long-term carbon storage and soil fertility through the oxalate-carbonate pathway. However, direct evidence showing that bacteria are taking fungal highways in nature is still missing.

The aim of this research is to identify environmental fungi-bacteria highways-like associations and to understand the frequency, diversity and activity of fungal highways in natural ecosystems.A new column-based isolation method has been developed to collect fungi-bacteria highway associations directly on the field. The columns were designed to avoid other bacterial dispersion mechanisms, including: transport by acarians and other soil organisms, spore dispersion, aerial dispersion and water dispersion. They also insure a minimal soil disturbance. The columns have been planted in Morocco, in soils under the influence of the oxalate-carbonate pathway, involving the presence of an oxalogenic plant (in this case *Opuntia fincus-indica*), fungi and oxalotrophic soil bacteria.

The associated fungi and bacteria will be identified, offering insights on the abundance and diversity of the fungi-bacteria highways-like associations. Their oxalotrophic and oxalogenic activities will be observed, and finally, the impact of the fungi-bacteria highway association on each organism activity will be measured by microcalorimetry. These results will help us to understand what kind of fungal highways associations are active in the soil, and how they are linked to the oxalate-carbonate pathway.

Niche-driven and geographically influenced patterns of diversity characterise euglyphid testate amoebae

Enrique Lara[1], Bertrand Fournier[1], Ludovic Roussel-Delif[2], Edward A. D. Mitchell[1]

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The analysis of DNA from environmental samples is revealing a huge environmental diversity of protists and other microorganisms. A new challenge is now to understand the factors that explain these diversity patterns. Here, we studied the diversity of a group of free-living testate amoebae, the Euglyphida, in forest litter and moss samples from a broad, worldwide sampling. We show that the diversity of euglyphid testate amoebae is clearly underestimated, reveal the existence of several novel clades, some of which contain organisms hitherto reported only from freshwater and marine environments. Soil (C/N ratio, pH), climatic, and biogeographical variables together explained a 28% of the observed diversity patterns in RDA, thus favouring a niche-driven community assembly, opposing previous expectations. Geographical distribution of the phylotypes was not random and suggested non-cosmopolitanism. Species richness was positively correlated to neutral pH, fast decomposition rates, warmer climates, and high annual precipitations. Our results contradict previous expectations on patterns of microbial diversity, and suggest that at least some microorganism communities' composition and diversity are ruled mainly like their macroscopical counterparts.

Estimation of the cloning biases in the evaluation of diversity in microbial eukaryotes: the case of the *Nebela tincta-bohemica-collaris* complex

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Estimation of the cloning biases in the evaluation of diversity in microbial eukaryotes: the case of the *Nebela tincta-bohemica-collaris* complex (Amoebozoa; Arcellinida; Hyalospheniidae)

Cultivation independent surveys based on environmental DNA are often used to screen eukaryotic diversity. Generally, these results have been considered as semi quantitative at best, because of biases in DNA extraction, PCR amplification or cloning. In this study, we compared the relative abundance of different species of testate amoebae from the *Nebela tincta-bohemica-collaris* complex in *Sphagnum* samples as observed under the microscope and as obtained by a cloning-sequencing strategy based on the mitochondrial cytochrome oxydase gene, subunit I (COI). Results show a similar composition in direct counting and clone libraries, once abundances are corrected by the biovolume of the amoebae (directly proportional to the number of mitochondria).

Euglyphida (Cercozoa; Rhizaria; Eukaryota) communities under pig cadavers by high throughput sequencing

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In forensic science, knowledge of the time elapsed between death and the finding of a corpse is a crucial body of evidence. To date, medical techniques such as observation of *rigor mortis* or evaluation of the degradation of certain proteins present in body fluids are used during the few days after death. Forensic entomology (i.e. estimation of PMI based on necrophagous fly larvae) is also routinely used. However, a weak point of these approaches is that their use is restricted to the few weeks following death.

It has been shown that the presence of a cadaver changes the physicochemical parameters (i.e. concentration of inorganic nitrogen and phosphorus) of the soil underneath for more than one year. In the present study, new PMI indicators were searched between the soil organisms community. For that purpose, the environmental diversity of Euglyphida was screened underneath three pig cadavers, three "fake pigs" (bags with an equivalent weight of soil) and three control sites, monitored during 2.5 years. A metabarcoding approach, based on Illumina sequencing of the v9 region of the SSU rRNA gene of all eukaryotes, was used and all sequences belonging to Euglyphida were sorted out. Results show that phylotype abundances were subjected to important variations in the control, and less so under the "fake pigs". Underneath the pig, most phylotypes declined from days 8 to 64, and then reached their initial level between days 309 and 1051. A new phylotype (nouv268, closely related to *Euglypha penardi*), in contrast, showed a progressive increase from day 64 to day 309, in all three replicates, and is thus a promising indicator for PMI.

Microbial interactions in the oxalate-carbonate pathway: fungal networks promote the activity of oxalotrophic bacteria

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The oxalate-carbonate pathway (OCP) involves biogeochemical processes at various ecosystem scales. In soils, oxalate produced either by plants or fungi forms poorly soluble precipitates of calcium oxalate (CaOx; Ksp 10-8.5). However CaOx does not accumulate. This is due to oxalotrophic bacteria that use oxalate as carbon (C) and energy sources, leading to a pH increase and eventually to $CaCO_3$ precipitation. Recent studies have demonstrated that efficient CaOx transformation and concomitant pH increase in soils were solely observed when both fungi and bacteria were present. This led us to hypothesize that fungi may promote CaOx bioaccessibility and biotransformation by allowing oxalotrophic bacteria to disperse on their mycelia in unsaturated environments ("fungal highway"-hypothesis). We therefore tested the influences of the motility of bacteria (flagellated vs. non-flagellated), the metabolic properties of fungi (non-oxalogenic vs.oxalogenic), the physicochemical surface properties (hydrophilic vs.hydrophobic) as well as the quality of the substrate (CaOx vs. malt media) on both the dispersal of bacteria along fungal mycelia and the CaOx turnover. We found that the presence of a mycelial network clearly enhanced both the dispersal of flagellated bacteria and CaOx turnover as compared to mycelial-free controls. The extent of the dispersal, however, depended on the experimental conditions chosen: The C-source, as well as the physicochemical surface properties of the medium strongly influenced the surface hydrophobicity of fungi and concomitant bacterial dispersal. Our study suggests that mycelial networks are relevant dispersal routes for oxalotrophic bacteria, hence increasing CaOx bioaccessibility and promoting a crucial function of an operating OCP, the oxalotrophic activity. It likewise highlights the importance of fungus-bacteria interactions as often overseen drivers of key microbial ecosystem functions in soil.

The amoeba *Nuclearia* sp. from Lake Zurich live in concert with ecto- and endosymbiotic bacteria

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Symbiotic interactions can be discovered throughout the phylogenetic tree of live. Culture independent molecular methods facilitate the investigation of close associations between protists and bacteria. More and more microbial players involved in different symbioses have been identified and characterized so far using methods like fluorescent *in situ* hybridisation (FISH) and sequencing of the 16S rRNA gene (respectively the 18S rRNA gene from the eukaryotic hosts). We investigated a naked filose amoeba isolated from Lake Zurich and the associated bacteria in culture. The amoeba was identified according to morphological characteristics and the phylogeny of the 18S rRNA gene. The unicellular organism shows typical features of the family Nucleariidae. It can have either a spherical (floating freely in the water column) or an amoeboid (attached to a surface) appearance. The morphology led to the affiliation of our isolate to the genus *Nuclearia*. Further we could show that the 18S rRNA gene of our nucleariid amoeba has a 99 – 100 % sequence similarity with *Nuclearia thermophila* (isolated from the 30 °C degree warm epilimnion of the Yunoko Lake in Japan). However, regarding the morphological characteristics, the nucleariid amoeba isolated in Japan and the amoeba from Lake Zurich differed considerably. N. thermophila is not associated with bacteria whereas the *Nuclearia* sp. from Lake Zurich harbours endosymbiotic bacteria inside the cytoplasm and undergoes additionally symbiotic relationships with distinct ectosymbiotic bacteria. One special pattern of ectosymbiotic bacteria attracted particularly our attention. *Nuclearia* sp. is surrounded by extracellular polymeric substances (EPS). Inside this mucous layer a highly regular arrangement of bacteria can be seen with the microscope. The full 16S rRNA cycle approach was used in order to identify these ectosymbionts. Endosymbiotic bacteria were investigated by transmission electron microscopy (TEM) and identified on the basis of general CARD – FISH probes. Further they were quantified in parallel with the growth of the amoeba. Our results suggest that the symbiosis between *Nuclearia* sp. and endosymbionts can be characterized as persistent and obligate whereas the association with different ectosymbionts is rather facultative and transient.

PyroTRF-ID: a novel bioinformatics methodology for the affiliation of terminal-restriction fragments using 16S rRNA gene pyrosequencing data

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The PyroTRF-ID bioinformatics methodology (http://bbcf.epfl.ch/ PyroTRF-ID/) was developed to combine pyrosequencing and T-RFLP for describing microbial communities and identifying T-RFs by comparison of experimental and digital T-RFLP profiles obtained from the same biological samples.
DNA extracts were subjected to amplification of the 16S rRNA gene pool, T-RFLP with the *Hae*III restriction enzyme, 454 tag encoded FLX amplicon pyrosequencing, and PyroTRF-ID analysis. Digital T-RFLP profiles were generated from the denoised pyrosequencing datasets. Sequences contributing to each digital T-RF were classified to taxonomic bins using the Greengenes reference database. The method was tested on bacterial communities found in chloroethene-contaminated groundwater samples and in granular biofilms from lab-scale wastewater treatment systems.
PyroTRF-ID was efficient for high-throughput mapping and digital T-RFLP profiling of pyrosequencing datasets. After denoising, multiple datasets comprising ca. 10'000 reads of 300-500 bp were processed in parallel within ca. 20 minutes on a high-performance computing cluster running on a Linux-related CentOS 5.5 operating system. Both digital and experimental T-RFLP profiles were aligned with maximum cross-correlation coefficients of 0.71 and 0.92 for high- and low-complexity environments, respectively. On average, 63±18% of all experimental T-RFs (30 to 93 peaks per sample) were affiliated to phylotypes.
PyroTRF-ID profits from complementary advantages of massive sequencing and T-RFLP in order to optimize laboratory and computational efforts for investigating microbial community structures and dynamics in any biological system. Massive sequencing provides high resolution in the analysis of microbial communities, and can be performed on a restricted set of selected samples. T-RFLP enables simultaneous fingerprinting of numerous samples at low cost and is adapted for routine analysis and follow-up of microbial communities on long term.

ORION (ORganic waste management by a small-scale innovative automated system of anaerobic digestION): a FP7 project on *in situ* biomethanization of specific wastes

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In Europe, 239 million tonnes per year of organic wastes are produced by restaurants, hotels, markets, fisheries and other small to medium size agro-food industries. The specific management of such wastes involves costly treatments and potential hygiene issues on-site. ORION aims at allowing most of these SMEs to manage *in situ* their organic waste in order to decrease the treatment costs and increase onsite hygiene conditions. Wastes will be valorised to produce bio-energy and a residue usable as a fertilizer. The scope is to develop digestion machines at the SME scale (1-50 m^3) for a large range of organic wastes, equipped with advanced control tools and sensors to reach an optimum reliability. ORION partnership includes potential end-users such as fisheries / aquaculture, hotel-restaurants, small agro-food industries, such as partners involved in the prototype design. They will rely on an interdisciplinary group of research centres in order to achieve the technical goal of the project. Preliminary studies will involve substrate analyses, possible substrate optimization using co-substrates and appropriate dilution, and digestion tests in bench-scale 1-step digesters managed semi-continuously. This will allow first predictions on the digestion yield, the stability of the operation and the composition of the residue, in view of the utilization of this latter as soil fertilizer, after appropriate stabilization, e.g. as compost. Then, a 650 litres pilot, using the patented technology developed by Digesto Sàrl will allow to test this technology with specific substrates, so as to precise the design of full-scale prototypes (3 – 30 m^3) to be installed at end-users place. Further assays will involve the nanotechnology group at CSEM, Neuchâtel, to study the effect of nanostructured surfaces either as bio-repellent or as biomass supports, to develop 2nd generation digesters using immobilized, structured methanogenic syntrophic biomass compatible with the treatment of particulate suspensions.

Identification of active oxalotrophic bacteria by BrdU labeled-DNA and their importance in the oxalate-carbonate pathway in natural environments

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The oxalate-carbonate pathway is probably one of the most important and underestimated potential carbon sink in terrestrial environments. To assess such biogeochemical process in tropical habitats, various elements have to be taken into consideration: the geology of the site, the presence of oxalogenic trees and fungi, and the presence of oxalotrophic bacteria. New insights into the diversity of culturable oxalotrophic bacteria have been obtained recently for three sites. However, studies focused on diversity using only the culturable fraction of the oxalotrophic bacterial communities are biased as this represents a small fraction of the total *in situ* diversity. In addition, isolation and molecular characterization of oxalotrophic bacteria are not necessarily informative of the active players into the oxalate-carbonate pathway. Therefore, the aim of this study is to assess the diversity of the active oxalotrophic bacterial communities using the BrdU DNA labeling technique in a microcosm system. The soil used has been collected in an oxalogenic system aside an Iroko tree in Cameroon. Microcosm treatments consisted of addition of calcium oxalate (0.5, 1 and 4% w/w respectively). Those were compared to an untreated control. After 12 days of incubation, a maximal pH value of 7.7 was detected in the treated microcosms (the initial pH was 6.4) due to oxalotrophic metabolism. At this time point, a DGGE profile was performed using both BrdU labeled and unlabeled soil DNA. The frc gene was used as molecular marker for oxalotrophy. Populations of Actinobacteria composed by the genera *Streptomyces*, *Kribbella* and *Nocardiopsis* were found as main groups of active oxalotrophic bacterial communities (48% of 65 sequenced DGGE bands). Those were followed by gamma and beta - Proteobacteria representing 19 and 13% of the active community, respectively. However, no difference in the community composition was observed when different concentrations of calcium oxalate were amended to the soil. This study highlights the relevance of Actinobacteria as the main members of the active bacterial community in the oxalate-carbonate pathway.

Microbial communities in geothermal sites. Are endospore-forming bacteria favored?

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Geothermal activity is observed in sites where geothermal energy is produced and stored, due to seismic and volcanic activity. Although these environments host microbial life, conditions for life in general would be characterized as unfavorable: high temperatures, acidic or alkaline pH, lack of nutrients, unfavorable oxygen or hydrogen levels, among others. As a response to these harsh conditions microorganisms have developed various survival strategies. We hypothesized that geothermal sites favor sporulation as a survival strategy and thus endospore-forming bacteria are abundant in microbial communities that inhabit such sites.

In order to evaluate this hypothesis, six geothermal sampling sites were considered: Lirima (Chile), a hot spring (Colombia) and Potamia, Thermia, N. Appolonia and Kanava natural hot springs (Greece). In addition, three geothermal stations in Gross-Shoenenbeck, Bruschal (Germany) and Soultz-sous-forets (France) were included. These sites are all habitats with temperature over 60°C.

DNA was extracted directly from sediments or fluids collected at these sites, using a method developed in our laboratory. A screening of the 16S rRNA gene and of the *spo0A* gene was carried out, in order to verify the presence of bacteria and specifically endospore-forming bacteria. Moreover, a qPCR method, also developed in our laboratory, was performed for both genes and a ratio was calculated in order to determine roughly percentage of endospore-forming bacteria in the whole bacterial communities. Finally, for description of endospore-formers in these communities, 454-pyrosequencing has been performed and comparison between the nine different sites has been made.

Endospore-forming bacteria are an important part in microbial communities that inhabit high-temperature environments. Environmental factors play a crucial role in the selection and abundance of these bacteria. Metagenomic studies based on functional genes could contribute to generate more information on the relationship between these bacteria and their environment.

Biogeography of soil Burkholderia populations

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The genus *Burkholderia* is an important component of soil microbial communities. *Burkholderia* species have a broad distribution in nature, occurring commonly in soil and in association with plants, fungi and animals, where mutualistic as well as parasitic interactions can be found. However, little is known about the factors influencing the abundance and diversity of *Burkholderia* species in their natural environment such as soil. Literature suggests that pH could play an important factor in shaping the biogeography of *Burkholderia*. To assess this question, two geographical scale sampling sessions were conducted. The global scale sampling consisted of soils collected across North and South America, whereas soils collected from an agricultural field (UK) represented the local scale sampling. A specific quantitative PCR (qPCR) protocol targeting *Burkholderia* 16S rRNA gene was developed to analyse the relative abundance *Burkholderia* in the above-mentioned soil samples. Results suggest that pH had a significant effect on *Burkholderia* relative abundance in soils at both sampling scales: high relative abundance was observed in acidic and moderate acidic soilsbut in alkaline soils, *Burkholderia* were under the detective limit of our method suggesting very low abundance or absence from these soils. Furthermore, *Burkholderia* relative abundance was increased in an soil microcosm study where soil pH was artificially acidified, suggesting that pH is an strong abiotic factor influencing *Burkholderia* relative abundance in soils. The diversity of soil *Burkholderia* populations was analyzed in a subset of 14 sites from the global scale sampling. Clone libraries targeting the 16S rRNA gene were constructed for each of the selected sites and a total of 675 sequences were obtained. Diversity analysis showed no correlation between pH and community composition, which was more influenced by factors linked to spatial distribution of samples and elevation of the sites. The most abundant and widely distributed species was *Burkholderia glathei* which made approx. 40% of all sequences. We are now investigating the factors underlying this preference of *Burkholderia* for low pH soils.

Alternative methods to control *Verticillium dahliae*: efficacy and impact on non-target soil fungi

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Verticillium dahliae is a soil-borne fungal plant pathogen causing, for instance wilt at strawberry, resulting in severe yield losses. Since the broad-spectrum fumigants methyl bromide and chloropicrin have been banned, alternative control methods need to be developed. Two such methods, biofumigation and anaerobe soil disinfestation (ASD), showed potential for control of *V. dahliae*. The first method is based on green manure releasing toxic isothiocyanates and the second on anaerobic soil conditions. Since the two methods possess an unspecific mode of action, other soil fungi could be affected. Hence, disturbance of non-target soil organisms should be minimal and should be investigated before widespread application of new control methods. The present study aimed at developing molecular-based diagnostic tools for *V. dahliae* and other plant pathogen species of the same genus and their application to assess efficacy of biofumigation, ASD and the soil fumigant metam potassium, in a sandy soil naturally infested with *V. dahliae*. Impact of the three treatments on non-target soil fungi was determined using Principal Component Analyses (PCA) on genetic profiles generated with fungal ribosomal intergenic spacer analyses (fRISA). The developed diagnostic tool allowed for a specific, sensitive and quantitative detection of *V. dahliae* and related plant pathogen species, with a considerable gain of time compared to cultivation-based method. Biofumigation had neither an effect on *V. dahliae* nor on other soil fungi. Metam potassium eliminated 91% of *V. dahliae*, but induced an increasing shift in fungal profiles after four, eight and sixteen weeks, which suggested a durable change of soil fungal communities. ASD appeared to be the most efficient treatment against *V. dahliae*, with an elimination of 98%. Shift in fungal profiles induced by ASD was maximal at the end of the treatment, i.e. after eight weeks, and decreased after sixteen weeks, suggesting a resilience of soil fungal communities. The efficacy of ASD against *V. dahliae* and its long-term effect on soil fungi and bacteria should be investigated at different field sites with different soil types.

Fungal control of dispersion and activity of bacteria in unsaturated environments

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In unsaturated porous media such as soils the dispersion of bacteria is greatly limited by the discontinuity and thickness of the water film. One mechanism explaining the movement of bacteria under these adverse conditions is the use of fungal hyphae as a dispersion network or the so-called "fungal highway" theory. The dispersion of bacteria by this mechanism has been studied in relationship to accessibility of substrates during the degradation of pollutants. However, the active role of fungi in the process has never been assessed. We studied the dispersion of GFP-tagged *Pseudomonas putida* on a dispersion network formed by the fungus *Morchella crassipes*. *P. putida* was able to migrate on the network colonizing the entire network in a few days and attaining populations of over 10^7 CFU after 2 days of incubation. However, using glass fibers with the same diameter as fungal hyphae led to the same result. In contrast, a more significant effect was observed for the fungus moiety of the system. Growth in the presence of bacteria modified the translocation of nutritional resources during the production of survival structures (sclerotia). We showed that *M. crassipes* uses a sophisticated mechanism to obtain advantage from the bacteria being transported in its network. The same sort of experiment was performed using other couples of bacteria and fungi. These experiments revealed that bacterial dispersion over the fungal network is a widespread phenomenon not linked to particular bacterial or fungal species. In addition, the effect of dispersion for the enhancement of the degradation of oxalate and other bacterial substrates was shown. The implications of this type of interaction are very significant considering the ecological and functional importance of bacteria and fungi for soil functioning.

Biopatinas or the use of fungi for the conservation-restoration of copper-based artefacts

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Alternative possibilities offered by biological treatments for the conservation-restoration of metal artworks are evaluated. Two research projects aim to modify existing corrosion products into more stable compounds, while maintaining the surface's original appearance. In the project of biopatinas, existing copper corrosion patinas were transformed into copper oxalates' patinas by *Beauveria bassiana*,a fungal strain isolated from vineyard soils highly contaminated with copper. The crystals aggregates were characterized through ESEM, FTIR and Raman microscopies, either on copper-enriched media or on corroded coupons[1-3]. Particular attention was devoted to the efficacy, durability and impact on color. The long-term behavior and performance of the biopatinas on corroded coupons were monitored and compared with reference materials (Cosmoloid H80, Dynasylan® F8263...) over a one-year exposure to atmospheric corrosion (ISMAR-SMS Genoa Harbor, corrosivity class 5). The measurements suggested a different weathering behavior of the biopatinas. Further scientific investigations are currently achieved in order to ascertain the parameters allowing the formation of a reproducible and homogeneous copper oxalates' patina. Based on the outcomes of this study, we will be able to propose a prototype that could be further develop as a user-friendly commercial kit for conservator-restorers. In the MAIA project, we propose to exploit the unique properties of some microorganisms for the stabilization of archeological iron. A synergetic microbial consortium will be specially designed for the formation of stable iron compounds, such as iron oxalates or magnetite, and the simultaneously removal of chloride ions that are the instigators of corrosion after excavation. A careful assessment of the methodology will be carried out over iron solid phases before real samples are integrated in order to validate the new conservation method elaborated.

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Is microbial diversity important for the functional performance of wastewater treatment plant microbial communities?

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Thousands of synthetic organic chemicals are used on a daily basis, many of which are increasingly being detected in surface and ground waters. These chemicals are typically present at concentrations in the ng to µg per liter range and are thus often referred to as micropollutants. To mitigate the possible risks of micropollutants, microbial communities are exploited to biotransform them from water supplies, such as in wastewater treatment plants (WWTPs). In order to predict how well a specific WWTP will biotransform micropollutants, it is important to determine whether biotransformation rates associate with specific ecological properties of the residing microbial communities. Theory predicts that the rates of community functions should be positively associated with community diversity. The objective of this research was to experimentally test this theoretical prediction.

We obtained ten independent microbial communities from ten different domestic and industrial WWTPs. We then added a mixture of ten structurally diverse micropollutants to each of the microbial communities and quantified biotransformation rates and identified biotransformation products by mass spectrometry. In parallel, we extracted total RNA from each community and sequenced 16S-rRNAs by pyrosequencing.

We found that the biotransformation rates of micropollutants that are oxidized are positively associated with both taxonomic richness and evenness, which supports theoretical predictions. However, we found that the biotransformation rates of micropollutants that are hydrolyzed are not associated with taxonomic richness or evenness, which raises caution about generalizing function-diversity relationships across different types of community functions. We hypothesize that these different outcomes are caused by how the different types of functions are distributed across different bacterial taxa, and we are currently performing experiments to test this hypothesis. In conclusion, our results reveal that function-diversity relationships do exist in WWTP communities, but depend on the specific type of community function.

Dietary iron supplementation of human gut microbiota associated rats impacts gut microbiota composition and metabolic activity

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Nutritional iron (Fe) deficiency is a global health concern and food fortification with Fe is a recommended strategy to correct Fe deficiency. However, the effects of Fe deficiency and supplementation on gut microbes and gut health are little known. The presented study investigated the impact of Fe supplementation on the gut microbial community and gut health of rats associated with a human gut microbiota.

Germ free rats (n=40) were housed in isolators for the entire trial period and inoculated with a human gut microbiota. Five groups of rats (n=8 each; control, iron depleted, iron depleted and repleted with 35 mg or 70 mg Fe/kg diet, iron oversupplemented with 70 mg Fe/kg diet) were fed a diet differing only in iron concentration during a depletion (13 weeks) and repletion (4 weeks) period. After sacrifice, cecal contents were analyzed on gut microbiota composition (qPCR) and metabolic activity (HPLC).

Unexpectedly, the iron status of rats (Hb, ferritin) did not change over the entire trial period despite high control on iron sources. However, cecal microbiota composition and metabolic activity were affected by Fe supplementation. 35 mg Fe/kg diet promoted dominant bacterial groups such as *Bacteroides* spp., *Clostridium* Clust IV members, *F. prausnitzii* and *E. hallii* while *Turicibacteraceae* were increased under a Fe deficient diet. More importantly, Fe supplementation increased butyrate production (24 ± 14 mM) by the gut microbiota 6-fold compared to rats receiving the control (3.3 ± 1.6 mM) or Fe deficient diet (4.8 ± 2.3 mM).

Our findings highlight the effects of dietary Fe on the microbial community of the cecal microbiota in rats. Fe supplementation increased beneficial gut bacteria while an iron deficient diet lead to a more pathogenic profile of the gut microbiota. Moreover, butyrate production of the cecal microbiota was increased with Fe supplementation. Butyrate has been attributed anti-cancer and anti-inflammatory effects and can act as energy source for colonocytes. Therefore, Fe supplementation might have beneficial effects on gut health.

New molecular method for the quantification of endospore-forming bacteria

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Bacterial endospores are highly specialized cellular forms that allow certain bacterial groups to tolerate harsh environmental conditions while conserving intact their genetic information. Recently, endospore-forming bacteria have been detected as dominant members of the microbial communities in polluted environments. In addition to natural habitats, EFB are often the cause of contamination problems in anthropogenic environments such as industrial production plants or hospitals. In order to achieve a better understanding of their role and prevalence in environmental and industrial fields a high sensitivity detection method is still needed. An approach based on qPCR quantification was therefore chosen and primer pairs have been developed.

Endosporulation is a complex mechanism that involved many regulatory genes. The major challenges of this work were: first, to find a functional gene specific for and common to all endospore-forming species; second, to identify conserved regions in this gene allowing primer design; third, to design primer sets suitable for qPCR; and, fourth to deal with degenerated bases and annealing specificity encompassing the diversity of endospore-forming strains. The gene spo0A was selected for this work. A new approach was developed based on multivariate analysis for primer design. The automation of such a design pipeline could be very efficient to target other functional genes in environmental communities.

Finally, the primer sets developed gave a reliable quantification when tested on different endospore-forming laboratory strains, but also in tests using environmental samples. Depending on the DNA extraction methodology used, a detection limit of around 10^3 to 10^4 cells per gram of initial material was calculated, giving this method a promising potential for the detection of endospore-forming bacteria in a wide range of applications.

Experimental community assembly of arbuscular mycorrhizal fungi in the field - SMRT ccs of tag-labeled multiplexed PCR amplicons

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Are communities of arbuscular mycorrhizal fungi (AMF, phylum Glomeromycota) recruitment limited? Are edaphic pre-adaptations important? Are successful immigrants phylogenetically distantly related to resident members? These are the questions I am trying to answer with a manipulative field experiment for which topsoil samples had been transferred and mixed into the local soils at eight distantly located grassland sites in the North and South of the Swiss Alps and on slightly acidic and alkaline soil. All experimental plots were planted to a uniform population of bait plants for later equal sampling of the (re-)assembled symbiotic AMF.

Choice of appropriate strategies for successful management and optimal usage of AMF in plant production and restoration ecology will heavily depend on whether AMF community assembly follows stochastic or deterministic trajectories. Moreover, if fungal traits were important, we need to know whether habitat conditions act as filters, or, whether rather overall trait similarity is limiting AMF co-existence via competition or antagonist-mediated negative interactions among assemblage members. Deterministic community assembly would make it possible to develop predictors of establishment success of inoculants among residents of the natural assemblages. Phylogenetic relatedness of potential immigrants and residents would be such a predictor, if there were phylogenetic trait conservation of the relevant fungal traits.

Here, I will first present pre-experimental data of the AMF assemblages in roots and soil from cloning and Sanger sequencing a nuclear rDNA marker showing some evidence for niche-based community assembly. Second, I will show findings of preliminary analyses of data obtained by Single Molecule Real-Time (SMRT) circular consensus sequencing (ccs) of tag-labeled multiplexed community PCR amplicons of a glutamine synthetase marker from 1.5 years after the set-up of the experiment pointing at strong recruitment limitation in natural AMF assemblages, but also suggesting that disturbance and competition-relevant traits play an important role. For discussion, I will put up crucial issues in acquiring and analyzing SMRT ccs.

Pyrosequencing based assessment of microbial communities in ice sediments and various depths along the Damma soil chronosequence

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Global warming has been causing the retreat of most European glaciers in the last years. Newly exposed barren soils are rapidly colonized by microorganisms and plants governing soil formation. Soil development and vertical stratification are primary factors influencing microbial community structures in these soils. However, little is known how microbial activity, diversity, and composition change with depth and how the vertical stratification reflects the relationships between nutrient contents and spatial distribution of microbial populations. This study aims at investigating the vertical distribution of bacterial and fungal communities in soils of different ages along a chronosequence of the Damma glacier forefield (Switzerland).

The surface, 5 cm and 20 cm deep soils of various ages and sediments on the top of the glacier were sampled. Microbial communities were analyzed by parallel pyrosequencing of bacterial and fungal ribosomal markers. Soil physicochemical parameters, microbial biomass, microbial activity, and relative frequencies of specific gene copies were related to changes in microbial community structure.

Bioinformatic processing yielded a total 270,549 and 280,394 bacterial and fungal pyrotags, respectively. The most abundant bacterial groups were the Betaproteobacteria (21%) and the Alphaproteobacteria (15%). The fungal community was dominated by Ascomycota (62%) and Basidiomycota (22%). The nutrient contents were highest at the surface and increased with soil age. Relative abundances of bacteria and fungi augmented with increasing nutrient concentrations in the soils. Furthermore, cyanobacterial abundance was highest in the top layer of barren soil and decreased along soil development.

In conclusion, the microbial communities are influenced by soil physicochemical parameters and vegetation development. Cyanobacteria thrive in the top layers of barren soils due to low nutrient contents and might provide a substantial part of the organic carbon at the early stage of soil development. Bacteria and fungi at all soil depths profit from increasing nutrient contents in more developed soils.

Validation of a novel in vitro fermentation model, PolyFermS®, for the swine proximal colon

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Accurate *in vitro* models are of high importance owing to ethical considerations and limitations of *in vivo* experiments. In this study we validated a novel in vitro intestinal model (PolyFermS®), inoculated with immobilized fecal swine microbiota, for efficient testing of feed additives.

A two-stage continuous fermentation model was developed, consisting of six reactors in series run with conditions of swine proximal colon. The nutritive medium was adapted to simulate the ileal chyme of swine fed on a standard cornstarch based diet. The first reactor (IR) was inoculated with 30 % (w/v) swine fecal microbiota immobilized in gel beads, and was used to inoculate five parallel test reactors. This set-up allowed constant inoculation (10%) of the test reactors with fermented effluent produced in IR. Effluent samples were tested for metabolite composition by HPLC and bacterial populations by real time PCR and pyrosequencing.

The PolyFermS® model maintained the bacterial composition of swine gut microbiota during the entire fermentation period of 54 days as demonstrated by stable 16S rRNA gene copy numbers of major bacterial groups used as stability indicators (*Bacteroides* spp., *Clostridium* Cluster IV, *Lactobacillus*/*Pediococcus*/*Leuconostoc* spp.). Moreover, bacterial diversity in IR remained stable over time as shown with OTU's (0.05) for d19/20 and d39/40 of 263 and 244, respectively.

A high and stable metabolic activity was observed with mean total metabolites concentrations in IR of 179.3 ± 6.3 mM during the entire fermentation period of 54 days. Thereof, acetate was the main metabolite detected (102.9 ± 7.6 mM), followed by propionate (44.8 ± 4.6 mM) and butyrate (20.5 ± 2.7 mM). Moreover, comparable metabolic activity and bacterial composition were tested in all parallel test reactors.

Our study indicates that PolyFermS® enables long-term and stable cultivation of swine gut microbiota, while maintaining a high bacterial diversity over time. This novel model could be suitable for parallel and efficient testing of dietary compounds or drugs on swine intestinal microbiota.

Microbial methane oxidation in the Arctic Ocean offshore Svalbard

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Methane (CH_4) is released in large quantities from ocean sediments by several different pathways, one of the most common being cold seeps. Aerobic methanotrophic bacteria in the water column consume a significant fraction of this CH_4, preventing CH_4emission into the atmosphere. Key environmental factors controlling the effectiveness of this biological CH_4filter are not well understood. In order to better constrain them, we investigated the temporal and spatial variation of aerobic methane oxidation rates (MOx) at active cold seeps off the coast of Svalbard. Water column methane concentrations were consistently high in bottom waters, up to 825 nM, and strongly decreasing towards the sea surface. We typically found the highest MOx rates, up to 3.1 nM/day, at 30 m above the sea floor, although the magnitude of MOx showed a high temporal variability despite the constant supply of CH_4. Comparison of the 2D distribution of MOx and water temperature revealed matching temporal patterns, indicating an oceanographic control on the magnitude of MOx: The warm Spitsbergen current meanders along the Svalbard continental margin, sweeping away Artic bottom waters, which contain a comparably large standing stock of methanotrophs. As a consequence, methane is injected into warmer water masses containing fewer methanotrophs, leading to a reduction in the magnitude of MOx. Our biogeochemical and oceanographic results will be completed by CARD-FISH, lipid biomarker analysis and stable isotope measurements of methane.

Characterizing iron and arsenate reduction of arsenate-reacted ferrihdyrite by *Shewanella putrefaciens* strain CN-32

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Mobilisation of solid phase arsenic under reducing conditions involves a combination of microbial arsenate and iron reduction and is affected by secondary reactions of released products. A series of model anoxic incubations were performed to understand the interaction between arsenate and ferrihydrite reduction by *Shewanella putrefaciens* strain CN-32 at different concentrations of arsenate, ferrihydrite, lactate and under conditions of given ΔGrxn for arsenate and ferrihydrite reduction under non-growth conditions. Under all experimental conditions, arsenate and ferrihydrite reduction occurred concurrently following addition of *S. putrefaciens* inoculums, suggesting no apparent competition between these two enzymatic reductions. A lag phase of ferrihydrite reduction was observed in incubations pre-equilibrated with ferrous ions, which was attributed to (bio)sorption of ferrous ions. The reduction kinetics of arsenate in ferrihydrite suspensions are controlled by the dissolved arsenate concentrations. At low arsenate to ferrihydrite ratios, arsenate reduction was very slow initially due to low aqueous arsenate levels; after several hours however, arsenate reduction accelerated as a result of arsenate mobilisation from the ferrihydrite surface through competitive displacement by reactive groups on the cell wall surface. Competition of lactate with arsenate for microbial contact slightly slowed down microbial arsenate reduction rates at high lactate concentrations. The favourability of microbial arsenate and ferrihydrite reduction rates did not correlate with ΔGrxn, suggesting their reduction kinetics was determined by other factors e.g. geochemical parameters.

Paleolimnology of Lake Geneva using endospore-forming bacteria

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Measurements of chemical and biological parameters, together with dating of the sediments, are used to understand the relationship between timelines of land-use change and the impact on ecosystem health. In this study, endospore-forming bacteria are used as a biological proxy to reconstruct the paleoecological history of Lake Geneva, Switzerland. We hypothesized that the ability to form endospores enables these bacteria to survive for long periods of time, therefore being a good proxy for paleolimnology.

A sediment core of 107 cm length was retrieved in the Rhone delta area. Mineralogical and sedimentological data were obtained from sections of 1 cm intervals. Two ^{137}Cs peaks were identified (1986 and 1964), indicating an average sedimentation rate of 1cm/yr. Thus, the core provided a record for the entire twentieth century. In parallel to physicochemical analysis, DNA was extracted from samples throughout the core (four cm depth intervals). The total number of bacteria was quantified by quantitative PCR using the 16S rRNA gene. The percentage of endospore-forming bacteria in respect to the total bacterial biomass at different depths was measured by quantification of the gene *spo0A*. This gene is coding for the sporulation transcription factor and specific for sporulating bacteria. Furthermore, a 600 bp fragment from this gene was subjected to amplicon sequencing in order to define endospore-forming OTUs and their phylogenetic distribution over sediment depth.

The results showed that despite the fact that the quantity of extracted DNA decreased with age of the sediment, the abundance of endospore-forming bacteria increased strongly over depth. This proved our hypothesis that these bacteria are indeed present in deep sediments. The diversity and abundance of this group of bacteria also changed significantly with depth and could be correlated to physicochemical parameters such as carbon to nitrogen ratio. This is the first time that endospore-forming bacteria are used as paleoecological proxies to reconstruct lake history based on molecular (culture independent) methods. We hereby successfully prove a new strategy for paleoecology using endospore-forming bacteria, which could also be applied to ocean sediments and longer sediment cores spanning longer timelines.

Antibiotic resistance as an emerging environmental contaminant

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There is mounting evidence that antibiotic resistant bacteria are released in large quantities from all sites of antibiotics application, e.g. with feces into the wastewater of hospitals and private households. Many of these bacteria enter wastewater treatment facilities where high concentrations of contaminants, high cell densities, and mixing with environmental bacteria provide a potential evolutionary hotspot for the selection, mobilization, and horizontal transfer of antibiotics resistance between potential pathogens and environmental bacteria. Resistant bacteria can establish in natural aquatic ecosystems or resistance factors can spread to natural populations by horizontal gene transfer, possibly increasing the natural resistance background, with unknown consequences for the likelihood of a transfer back into clinically relevant bacterial strains. We studied the prevalence of resistant bacteria and various resistance genes in the wastewater stream of Lausanne and the receiving water body, the Vidy bay of Lake Geneva. Using a combination of cultivation based and quantitative molecular methods, we could show that resistant bacteria, including highly multiresistant strains, are only insufficiently eliminated by the current WWTP. We show that qPCR can be used to quantitatively track antibiotic resistance genes in the bay sediments and found clear signs of local contamination originating from the wastewater discharge. Other data from various Swiss Lakes show the variability of resistance gene abundance and indicate an influence of WWTPs but not of the input from the agricultural sector. Prevalence of resistance genes did not correlate with microbial community structure. Antibiotic resistance is an emerging environmental contaminant in Switzerland with unique properties and yet undefined risk that needs to be further investigated.

Poster presentations

Corrinoid auxotrophy in the obligate organohalide respiring *Dehalobacter restrictus*

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Corrinoids (derivatives of vitamin B_{12}) are an essential cofactor of reductive dehalogenases, the key enzymes involved in the environmental-friendly process of organohalide respiration (OHR). However, genomic and physiological analyses of obligate OHR bacteria (such as *Dehalobacter* and *Dehalococcoides*) have delivered contrasting results on the ability of de novo corrinoid biosynthesis. This raised the question of the source of corrinoids for obligate OHR bacteria in the environment and their biosynthesis/scavenging mechanism of corrinoids.

Dehalobacter is an important bacterial genus for the bioremediation of organohalides such as chloroethenes, chloroethanes and chloroform. Genomic analysis of *D. restrictus* revealed the presence of all the genes required for the production of corrinoids (1), however the strain is incapable of de novo biosynthesis. The general aim of the present study is to better understand the corrinoid metabolism of the genus *Dehalobacter* at the level of biosynthesis, regulation and transport.

A detailed analysis revealed that the *cbiH* gene whose product is involved in the corrin ring contraction displays a frame-shift mutation which was confirmed experimentally, suggesting that it might represent a possible checkpoint behind the corrinoid auxotrophy. Moreover, in proteomic data of a *D. restrictus* culture growing in presence of vitamin B_{12} in the medium, several corrinoid biosynthesis proteins were not detected arguing for specific regulation mechanisms. The transport and scavenging metabolism of corrinoid by *D. restrictusis* now under scrutiny.

(1) Rupakula et al. (2013), Philos. Trans. R. Soc. B, in press.

How can plant-associated pseudomonads with antifungal activity become insect pathogens?

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Root colonizing fluorescent pseudomonads are well known for their ability to improve plant growth by the suppression of soilborne diseases. These bacteria produce a wide array of antifungal compounds they use as weapons to protect roots against the attack of plant-pathogenic fungi. To our surprise we detected some years ago in *Pseudomonas fluorescens* CHA0, a strain with well-described antifungal activity, a genomic locus encoding a protein with high similarity to the Mcf insect toxin produced by *Photorhabdus luminescens*. This protein, termed Fit, represents a novel insect toxin in root colonizing pseudomonads. Indeed, CHA0 exhibits potent oral activity against larvae of major lepidopteran insect pests, when sprayed on plant leaves and has the capacity to multiply and persist within insects. This finding prompted us to investigate the occurrence, abundance and origin of insect pathogenicity in plant-associated pseudomonads. To this end we performed a PCR based screening on a large collection of pseudomonads isolated not only from plants but from different environments followed by sequencing and phylogenetic analysis of Fit producing Pseudomonas strains in order to reconstruct the evolutionary history of the Fit toxin gene and to analyse its mode of evolution. Our results revealed that Fit is present in *Pseudomonas chlororaphis* and in a small subgroup of fluorescent pseudomonads producing the antifungal compounds 2,4-diacetylphloroglucinol and pyoluteorin. We found this group to be closely associated with *P. chlororaphis* when analyzing both, four housekeeping and the nucleotide sequences of the Fit toxin gene. In addition, a more detailed sequence analyses suggest that Fit was acquired by horizontal gene transfer from entomopathogenic *Photorhabdus* with subsequent rearrangements of the toxin cluster. Testing our *Pseudomonas* collection for insecticidal activity revealed that only strains harboring Fit are toxic to insects.

In summary we identified a genetically distinct subgroup of biocontrol pseudomonads as exclusive carriers of the Fit toxin gene, a marker for insecticidal activity of plant growth-promoting pseudomonads. Our findings suggest that a specific group of root-associated pseudomonads acquired a potent insect toxin enabling them to kill insects and colonize a new ecological niche.

Bacteria as biocontrol agents of phytopathogenic fungi: the role of volatile organic compounds

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Many soil borne bacteria emit compounds, which promote plant growth and/or inhibit phytopathogenic fungi. As recent studies indicate, volatile organic compounds (VOCs) might play a significant role in these interactions between bacteria and fungi. Plant diseases caused by fungi such as late blight (*Phytophthora infestans*) in potatoes or snow mould (*Microdochium spp.*) in small-grain cereals cause major crop losses in sustainable agricultural systems as organic production, where effective plant protection strategies are scarce. In this work we isolated bacteria from the rhizosphere and necrotic shoot tissues of late blight infested potato plants. Isolates were then investigated in vitro for direct and VOCs-mediated antagonistic effects, using a co-cultivation and a divided Petri dish assay. We found several strains, which inhibited growth of *P. infestans* and of *Microdochium spp.*, some of which produced inhibiting volatiles. The next important steps will be to determine which volatiles are responsible for the antagonistic activity and to test the effects of the promising bacterial strains and/or molecules *in planta*.

Pelletization of Micro-algae by Induced Lichen Formation through Co-culture with Filamentous Fungi

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The use of micro-algae within industrial applications, such as biofuel production, has several limitations with regard to cost and therefore sustainability. Micro-algae biomass consists of small single cells that stay in suspension making them difficult to harvest, requiring filtration, centrifugation or flocculation methods which contribute to a significant cost of the total biomass production. Recent studies have investigated the use of co-cultivation of fungi and algae to form symbiotic lichen communities as a mechanism of harvesting the micro-algae biomass.Lichens are symbiotic community structures comprised of fungal mycobionts and algal photobionts. Algae, inoculated with fungal biomass interact to form a pellet structure. Pelletization has several beneficial effects for industrial application due with regard to increase biomass and efficient harvesting by size exclusion. Additional benefits of lichen formation would be to bio-remediate wastewater as well as potentially extract valuable by-products such as carotenoids.

Three commercially interesting algal species, namely *Chlorella vulgaris*, *Chlorella sorokiniana* and *Scenedesmus vacuolatus*, has been investigated for their compatibility with a previously uncharacterized *Sordariomycete* to form a symbiotic lichen structure. To achieve optimal culture conditions parameters such as pH and hetrotrophic culture has been considered, photobiont-mycobiont interaction and biomass composition has been evaluated for potential biotechnological application.

Testing plant growth promoting rhizobacteria (PGPR) isolates as inoculants for Ilex paraguariensis (yerba mate)

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Trade of yerba mate (*Ilex paraguariensis*) is a very lucrative business in Argentina, Paraguay, and Brazil. In the province of Misiones, Argentina, the area covered with highly productive plantations corresponds to approximately 173,000 ha. However the increase of low productive plantations (currently 70.000 ha) due to soil degradation is a major concern. One of the strategies to improve soil quality and fertility is to use cover crops like *Phaseolus vulgaris* to obtain mixed plantations. The aim of this project is to assess an alternative approach by studying the role of native PGPR as potential inoculants, in order to improve productivity of yerba mate in low productive plantations. After screening for *in vitro* PGPR activity, three strains isolated from yerba mate plantations were selected. The isolates YD4, YC2 and YC3 were characterized and assigned to the genera *Burkholderia*, *Rhyzobium* and *Agrobacterium*, respectively. These three strains were used as inoculants for plant assays under controlled conditions. *In vitro* inoculation of yerba mate dormant seeds did not promote the germination. Rudimentary embryos were then cultured *in vitro* to obtain seedlings for inoculation assays. However only 2% of embryos developed to normal plants. Due to the difficulty to obtain yerba mate plants in vitro, the inoculants are currently tested in a microcosm assay in Argentina. In order to test the effect of the PGPR inoculants in others crops, *Phaseolus vulgaris* plants were inoculated, irrigated with different concentrations of a nutrient solution and harvested after 30 days to measure dry shoot mass, dry root mass and specific foliar area (SLA). The assay with *Phaseolus vulgaris* plants showed that inoculation with isolate YC2 had a significant effect (P¡0.05) on the specific foliar area (SLA) in the two lowest nutritive concentrations (0.25X and 0.125X) according to Tukey's comparison test. However, no significant effect was observed on the dry shoot mass and dry root mass. In conclusion the difficulties to germinate and propagate yerba mate in vitro limited the test of PGPR inoculants under control conditions. However, the relevance of testing plant inoculation with PGPR in low fertility soils was confirmed by the positive effect of YC2 inoculation on the specific foliar area of *Phaseolus vulgaris* at low nutrients conditions.

Predator-prey interactions induce rapid adaptation in a freshwater bacterial isolate

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The foraging of size-selective protistan grazers is an important factor in the aquatic environment that shifts bacterial community composition towards protected species and might select for specific adaptations in bacteria. As a result, flagellates continuously influence the evolution of bacteria, and bacterial genotypes with increased antipredator fitness should evolve. The formation of aggregates is one such strategy that increases the ability to escape flagellate predation. We investigated the adaptation of an aggregate forming bacterium isolated from Lake Zurich, *Sphingobium* sp. Z007, to constant grazing pressure by the bacterivorous flagellate *Poterioochromonas* sp. strain DS. To assess evolutionary changes of the bacterial strain we ran a long-term experiment with semi-continuous batch cocultures of predator and prey in oligotrophic medium for a period of several months. Phenotypic shifts, such as the ability of aggregate formation, biofilm development, and substrate degrading abilities of *Sphingobium* strains evolved with and without flagellates were examined every six weeks. Strains evolved under predation pressure showed a continuous increase in aggregate formation. This putative fitness gain in coexistence with *Poterioochromonas* was, however, accompanied by lower growth rates and cell densities compared to strains evolved without predation pressure. These results are pointing at the importance of protistan predation as a driver of evolutionary changes in aquatic bacteria.

Identification of Biomass Utilizing Bacteria in a Carbon Depleted Glacier Forefield Soil by the Use of ^{13}C-DNA-Stable Isotope Probing

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As Alpine glaciers are retreating rapidly, bare soils composed of sand, gravel and stones, with low organic C and N contents, are becoming exposed. Carbon availability is a key factor regulating microbial diversity and ecosystem functioning in these soils. The aim of this study was to investigate how bacterial activity, community structure and diversity are influenced by organic carbon availability. Bare soils were supplied with ^{13}C-labelled fungal (*Pencillium* sp.) and green algal (*Chlorella* sp.) biomass and incubated at 4°C and 18°C. These organisms have previously been isolated near the glacier terminus. CO_2 evolution from soil and its δ^{13}C signature were monitored up to 60 days. DNA stable isotope probing followed by T-RFLP profiling of 16S rRNA genes was employed to identify bacteria able to assimilate carbon from these biomass amendments.

The carbon resource clearly influenced the composition of the bacterial communities in the soil and the ^{13}C-labelled microbial biomass were differently incorporated. *Flavobacterium* sp. within the *Bacteroidetes* predominantly incorporated fungal-derived C whereas the algal-derived C was mainly incorporated by *Acidobacteria* and *Proteobacteria*. Higher respiration and higher bacterial activity indicated a more efficient utilization of algal cells than fungal cells. Moreover, we observed that the incubation temperature also had an effect on the consumers.

This study emphasizes the important role of both fungal as well as algal cell fragments in increasing the available carbon pool in recently deglaciated bare soils. We found that only 20% of C was respired as CO_2 after 60 days, and the rest, we presume, remained in the soil, increasing the soil organic matter content.

The Influence of Agricultural Management Practices on the Soil Microbiome as Revealed by Massively Parallel Pyrosequencing

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Since 1978, the DOK long-term agricultural management experiment compares biodynamic, bioorganic, and conventional farming practices and provides an excellent resource to assess farming-related effects on soil quality characteristics. Previous work based on genetic profiling of fungal and bacterial markers has demonstrated that different farming systems select for specific microbial communities. These data have revealed consistent effects of long-term farmyard manure application and short-term crop cultivation. A classical sequencing approach yielded indications for specific bacterial groups that were characteristic for specific management practices; however, limitations inherent to these first-generation sequencing techniques did not allow for sufficient diversity coverage and sample throughput in order to determine robust management indicators. Recent developments in sequencing technologies stimulated a reassessment of this site at much higher resolution by reanalyzing the same soils using massively parallel pyrosequencing of bacterial and fungal ribosomal markers.

Based on samplings from two different years, we screened the structure of the soil microbiome in five different farming systems each represented by two different stages in the crop rotation (= 80 soil samples). Bioinformatic processing yielded a total of 594,340 and 523,928 bacterial and fungal pyrotags corresponding to 3877 and 2554 operational taxonomic units, respectively. Actinobacteria (30%), Proteobacteria (29%), Acidobacteria (10%), Bacteroidetes (9%), and Chloroflexi (6%) as well as Ascomycota (53%), Basidiomycota (17%), and Mucoromycotina (17%), were the predominant groups in these soils. In-depth pyrosequencing generally supported results from the previous profiling studies, but provided increased resolution of farming-related effects on soil microbial community structures by identifying certain effects that went undetected using the traditional lower-resolution techniques. In particular, fertilization and crop effects on the fungal community were more pronounced. Taxa-treatment association analysis revealed bacterial and fungal groups that are characteristic for specific farming practices and their functional significance for soil quality can now be further addressed.

Degradation of raffinose by a novel strain of *Pseudomonas*

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A Gram-negative, chemoorganotrophic, cyanide forming, flagellated, rod-shaped bacterium (strain CCOS191) was isolated from soil. CCOS191 was phylogentically characterized applying 16S rRNA and MLSA (multilocus sequence analysis) gene sequence similarity as well as by DNA-DNA hybridization and revealed to be a novel species within the genus *Pseudomonas*. Closest relatives were *P. mosselii* and *P. entomophila*. Carbon source utilization testing with BiOLOG Phenotype MicroArrays and subsequent comparison with ten related *Pseudomonas* species by principal component analysis showed a very special metabolic feature – which makes CCOS191 unique – namely the utilization of raffinose, a trisaccharide consisting of glucose, fructose, and galactose. Although earlier very few pseudomonads have been described as being able to utilize raffinose as sole carbon source, most of the strains have been reclassified and renamed. Today, only a few strains of *P. syringae* – but not all – can grow on raffinose as sole carbon source. For the microbial degradation of raffinose, the activity of alpha-galactosidase is required. Only very few *Pseudomonas* species are known to produce this enzyme, e.g., *Pseudomonas atlantica* (a marine bacterium, reclassified as *Alteromonas atlantica* and later as *Pseudoalteromonas atlantica*). However, *P. atlantica* was described as not being able to utilize raffinose, although alphagalactosidase activity was observed. In our case, the closest phylogenetic relatives of CCOS191 (*P. mosselii* and *P. entomophila*) were not able to grow at all on raffinose. In summary, genotypic analysis in combination with physiological testing clearly demonstrated that strain CCOS191 represents a novel species within the genus *Pseudomonas* for which the name "*Pseudomonas raffinosivorans*" sp. nov. is proposed.

Genetic tools for genotyping, detection, and quantification of *Metharizium* species in soil

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Entomopathogenic fungi of the genus *Metharizium* constitute an important biotic component in the natural regulation of arthropod populations including agronomically important pests. Due to their ability to infect and control insect pests, these fungi have a considerable potential as biocontrol agents (BCAs). For the development and application of a BCA, profound knowledge for instance on its host and habitat type dependent occurrence, population structure or possible non-target effects is required. Availability of efficient tools that allow genotyping, detection, and quantification of the BCA is crucial when addressing such aspects. The goal of this study was firstly to assess an existing genotyping tool, which is based on 41 SSR markers isolated from *M. brunneum*, *M. robertsii* or *M. anisopliae* for transferability to other *Metharizium* species, i.e., *M. pingshaense*, *M. majus*, *M. guizhouense*, *M. lepidotiae*, *M. acridum* and *M. globosum*. The second goal was, to develop a cultivation-independent detection and quantification (qPCR) tool for *Metharizium* species in soil. Assessment of the transferability of the 41 SSR markers revealed that 39 markers can be transferred to other *Metharizium* species, i.e., they can be amplified from species they were not isolated from and are polymorphic. Furthermore, 1 marker was specific for the species from which it was isolated. A specific PCR for detection of *M. robertsii* was developed and promisingPCR primers specific for *M. brunneum* were designed using specific sequence signatures in the ribosomal intergenic spacer region (rIGS) of the nuclear ribosomal RNA gene cluster. In a next step, the specificity of the PCR primers designed for *M. brunneum* will be tested and both *M. robertsii* and *M. brunneum* specific PCRs will be optimized for qPCR. For detection and quantification of other *Metharizium* species like *M. anisopliae* and *M. pinshaense*, additional loci will be assessed to find species specific signatures that allow specific PCR primers design.

New methods to quickly screen diversity of endospore-forming bacteria in environmental samples

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Endospore-forming bacteria (EFB) are Gram positive bacteria belonging to the phylum Firmicutes. They are ubiquitous in most environments but have been mainly studied in medical and biotechnology research. The diversity and function of EFB in the environment therefore is poorly known. The transcription factor for initiation of the endospore-forming process encoded by the gene *spo0A*, has been assessed as a marker to target EFB previously in our laboratory. *spo0A* primers were tested on over 100 EFB isolates to validate their specificity and were then used to characterize the abundance of EFB in environmental samples by real-time PCR and the community composition by pyrosequencing. However a raping fingerprinting method to determine the diversity of EFB in environmental samples is still missing. A database of 186 *spo0A* sequences of about 200 bp was constructed from EFB isolates and sequences available from JGI/IMG database. The sequences GC content is in the range of 35-65% and there is a high diversity in sequence composition. In this study, two different fingerprinting methods (DGGE and T-RFLP) have been assessed *in silico* by using this database. The development of these methods to characterize the community structure and diversity of EFB in environmental samples is presented here. These methods provide a rapid screening approach to study bacterial communities from environmental samples. They are easy-to-use and demand little time, thereafter are ideal as initial overview methods when handling high number of samples.

Experimental confrontation of natural arbuscular mycorrhizal fungal assemblages in the field - Study of community assembly

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A field experiment had been set up three years ago with plots at eight distant grassland sites in Switzerland, chosen to be located in two different biogeographic regions (North and South of the Swiss Alps) and on soils either slightly acidic or alkaline for maximal distinctiveness of the indigenous arbuscular mycorrhizal fungal (AMF) assemblages. Topsoil samples from each site had been reciprocally transferred and mixed into the local soil of experimental subplots, which were afterwards planted to a uniform population of bioassay plants (*Plantago lanceolata*), for recording the community assembly process when indigenous AMF are confronted with foreign ones. Events of immigration will be evaluated on the background of possible functional difference as suggested by phylogenetic distinctiveness, assuming phylogenetic trait conservatism. We expect to find foreign AMF strains from sites of a similar soil pH, that are distantly related to resident ones, as most frequent immigrants, owing to reduced environmental filtering and differences in trait sets. If confirmed, the measure of relative phylogenetic relatedness of AMF inoculants to known site-adapted resident strains could be used as a generally applicable predictor of establishment at new introduction sites. Knowledge about whether natural AMF assemblages are recruitment limited or not and about the relative importance of environmental filtering versus limiting similarity will tell us which plant growth and health-stimulating AMF inoculants to apply. Furthermore, a better understanding of AMF community assembly will help us to foresee knock-on effects of climate change on natural AMF assemblages and to assess risks of biotic homogenization as a consequence of enhanced propagule dispersal via modern human activity, including release of non-indigenous AMF inoculant strains.

Characterization of the metallo-cofactors of the tetrachloroethene reductive dehalogenase purified from *Sulfurospirillum multivorans*

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Sulfurospirillum multivoransis an epsilonproteobacterium able to grow anaerobically with hydrogen as electron donor and tetrachloroethene (PCE) as terminal electron acceptor (organohalide respiration). The dechlorination is mediated by the PCE reductive dehalogenase (PceA), which is a corrinoid-containing iron-sulfur protein [1]. This enzyme is located at the periplasmic face of the cytoplasmic membrane [2] and represents the terminal oxidoreductase of a so far uncharacterized membrane-associated respiratory chain. The corrinoid cofactor of PceA was shown to be Norpseudo-B_{12}. However, until now, only little is known about the role of the iron-sulfur clusters in the electron transfer within the enzyme.

In this study a mutant strain of *S. multivorans* producing an affinity-tagged PceA was characterized with regard to growth and the ability to dechlorinate PCE as terminal electron acceptor. A simplified protocol for PceA purification was established and optimized for a fast and efficient isolation of pure and homogenous recombinant PceA in high amounts. The iron content and the number of corrinoids per molecule PceA were quantified. Using electron paramagnetic resonance spectroscopy (EPR) and concomitant redox titration, the type of the Fe-S clusters and their midpoint redox potentials were determined.In addition, the midpoint redox potentials of the corrinoid cofactor were measured and compared to already published data [3]. From the experiments presented here a tentative scheme of the electron transport pathway in the PCE respiratory chain of *S. multivorans* was derived that combines recent biochemical and spectroscopical results.

[1] Neumann et al. (1996). J Biol Chem 271: 16515-16519
[2] John et al. (2006). Arch Microbiol 186: 99-106
[3] Kräutler et al. (2003). Helv Chim Acta 86: 3698-3716

Functional genotyping of *Sulfurospirillum* spp. in mixed cultures allowed the identification of a new PCE reductive dehalogenase

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Chlorinated compounds are widespread soil and groundwater pollutants. Because of industrial activities, large amounts of chlorinated ethenes were discharged into the environment over the last decades. The underlying biodegradation process, organohalide respiration (OHR), is a bacterial anaerobic respiration in which the chlorinated compounds, such as chloroethenes, are used as terminal electron acceptors. The key catalytic enzyme in OHR is the reductive dehalogenase (RdhA). SL2-PCEb, an enrichment culture dominated by *Sulfurospirillum* spp., was shown to stepwise dechlorinate PCE to trichloroethene (TCE) and *cis*-dichloroethene (*cis*-DCE), suggesting the successive involvement of multiple populations. However neither T-RFLP on 16S rRNA genes nor the analysis of the 16S-23S intergenic spacer (ITS) allowed identifying distinct *Sulfurospirillum* strains (1).

Recently, two subcultures were derived from SL2-PCEb showing distinct dechlorination potential: SL2-PCEc which dechlorinates PCE to TCE only, and SL2-TCE which was selected on TCE but kept the potential to dechlorinate both PCE and TCE. As genotyping based on rRNA genes was not possible here, cloning/sequencing of *rdhA* genes and a T-RFLP method dedicated to distinguish the functional genes were successfully applied allowing the identification of a new RdhA from *Sulfurospirillum*, $\mathrm{PceA}^{\mathrm{TCE}}$, involved in the dechlorination of PCE to TCE exclusively. This new subculture, SL2-PCEc, is now the focus of several new investigation lines which will be also presented.

(1) Maillard et al., 2011, Biodegradation 22:949.

The potential application of Amanita muscaria in vanadium bioremediation

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Vanadium contaminated soil and solid waste is increasingly becoming an environmental problem. V has numerous benefits in iron and steel production, particularly in atomic energy industry, aircraft construction, and space technology. From a physiological point of view, V is a nutrient element in very small amounts for human beings, plants, and animals, but it is also well known for its toxicity. Threshold toxicity for V is near 10 mg per day in humans. Nevertheless, V is also a therapeutic agent to prevent and treat diabetes mellitus. A survey of V concentrations in a number of medicinal plants shows that these plants contain high amounts of V, especially in leaves and aerial parts. Aqueous extracts are used as pharmaceutical formulations in phytotherapy. Besides plants, a series of fungi can take up heavy metals very efficiently resulting in high intracellular concentrations. In particular, *Amanita muscaria* (commonly known as fly agaric) is able to accumulate high amounts of V, especially in the fruiting body. Vanadium is present as a unique organo-metallic complex, amavadin. This immediately raises the question whether we can use *A. muscaria* to treat with V-containing solid waste. Until today, such an application has not been studied yet. At the same time, ectomycorrhizal fungi play an important role in plant adaptation to contaminated soils. As example in the literature, *A. muscaria* inoculation significantly changed growth and heavy metal (Cd, Cu, Pb, Zn) uptake of the willow clones. As preliminary work, we sampled a series of *A. muscaria* specimens and determined the V content in various parts of the fruiting body in relation to the V concentration in bulk soil. By cultivating mycelia in the presence of V-containing solid wastes and soils, the mobilization, extraction, and recovery of V might be possible.

Removal of Copper (Cu) from Industrial waste water using *Bacillus* spp.: An economical and viable approach

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Untreated industrial waste is a major source of pollution in the environment and is basically composed of toxic levels of organic and inorganic compounds. Metal contaminants such as Cd, Cu, Pb, Hg, Cr and Zn form a major part of the industrial effluents. Copper (Cu) is one of the most toxic heavy metals and is deleterious for both human and animal health. Bacteria exposed to high levels of heavy metals in their environment have adapted to this stress by developing various resistance mechanisms and could be utilized for the detoxification and removal of heavy metals from polluted effluents. Biosorption is an effective heavy metal removal mechanism using resistant microorganisms and we hypothesize that gram-positive endospore forming bacteria (*Bacillus* spp.) have stronger sorption capabilities towards heavy metals than gram-negative bacteria. The main objective of this study is to test the removal of Cu from an industrial effluent using *Bacillus* spp. thorough different approaches. A total of 104 strains of Gram-positive aerobic endospore-forming bacteria related to *Bacillus* spp. from different world wide natural environmental samples were isolated, characterized and tested for copper resistance with different concentrations (0.5-3.0mM). The tolerance of these microorganisms decreased with increasing copper concentrations. Four highly resistant strains, tolerating up to 3 mM of copper, were identified as *Bacillus* spp. based on their 16S rRNA gene sequence. Biosorption studies were carried out using vegetative cells, spores, and inactive cells (killed and freeze dried) for both Cu aqueous solution and an industrial effluent. The effects of various parameters such as optimum biomass, contact time, and Cu concentrations on Cu removal efficacy were investigated. Under copper stress, the strains had a relatively high mean specific growth rate and exhibited a high degree of bioaccumulation ability. With the advantages of high Cu uptake capacity, satisfactory recovery efficiency and high Cu tolerance suggests that *Bacillus* spp. can be used as effective adsorbents for the removal and recovery of copper from industrial wastewater.

IMPLEMENTATION OF A GENETIC TRANSFORMATION STRATEGY TO IMPROVE THE BIOLOGICAL CO_2 CAPTURE IN *CHLORELLA* SP. AND *SCENEDESMUS* SP.

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The concept of producing renewable microalgae biomass using as a main nutrient source a secondary wastewater stream is presently under investigation. It is planned to purify the wastewater by the growth of algal biomass while producing a feedstock that can be used for the bioenergy sector. If important biomass density is achieved, this strategy will be adopted in a novel process named SunCHem (proposed at the EPFL-PSI SWT group) using hydrothermal treatment of microalgae to produce methane. By coupling the wastewater treatment, the capture of CO_2 emissions and the production of methane through microalgae, we directly impact the sustainable use of carbon resources. In this work, we aim to develop a strategy for the genetic improvement of microalgae in order to enhance the accumulation of carbon inside the cells. The strategy contributes to efficiently obtain a significant change in the production of algal biomass and thus potentially, to increase the methane production yields. The strains *Chlorella* sp. and *Scenedesmus* sp. have been used to apply the modification of genes involved in these metabolic pathways; in particular, the genes codifying for the glutamine synthetase (*glnA*). *Chlorella* sp. was isolated from the wastewater treatment plant and adapted for axenic culturing. These microalgae were also selected due to an appropriate carbon balance for their future hydrothermal conversion into methane.

Metagenomic analyses of PCB-degrading consortia present in sediment-free microcosms revealed novel microbial structures

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In anaerobic habitats, organohalide respiration (OHR) of polychlorinated biphenyl (PCB) congeners was reported to be a crucial step in achieving their elimination. The goal of the present study was to identify members of the microbial consortia involved in the degradation of these congeners in anaerobic sediment-free microcosms (SFMs). To do so, inert silica particles were amended with 20 ppm of PCB congeners (Delor 103) in a mineral medium. SFMs were inoculated with bacterial consortia enriched from river sediments sampled near the PCB-polluted Chemko Strazske site (Slovak Republic). Bromoethanesulfonate (BES) was added to a subset of the SFMs so as to inhibit the growth of methanogenic *Archaea*. Analysis of the OHR consortia involved microscopy, T-RFLP analysis on total DNA extracts, and metagenomic analysis on rRNA samples. PCB congeners were quantified with GC-ECD.
T-RFLP analysis on samples taken at regular time intervals showed a clear change in the community structures over time, delineating a net adaptation process to the growth conditions imposed to the SFMs. Very divergent bacterial community structures were found, although all microcosms were grown under standardized conditions. After almost one year of cultivation, total congener degradation varied between 11.9% and 55.2%. In SFMs without BES, metagenomic analysis revealed the presence of obligate OHR bacteria (*Dehalococcoides* sp., up to 31,5% of the sequences) and facultative OHR bacteria (*Desulfuromonas* sp., up to 13%; *Desulfitobacterium* sp., up to 4,8%). No sequences matching with other OHR bacteria know to degrade PCB congerners, such as *Dehalobium chlorocoercia* DF-1 and bacterium o-17 were present. As expected, the addition of BES strongly reduced the *Archeae* diversity. It also clearly induced shifts in bacterial diversity, resulting in unusual community patterns, as well as the apparent strong decrease of the abundance of *Dehaloccoides*-related sequences. Furthermore, BES-amended SFMs revealed an unusually high abundance (up to 21.8%) of sequences closely affiliated with *Geobacter* spp. with a concomitant high percentage of PCB degradation (up to 44.3%). These findings indicated that bacterial populations other than *Dehaloccoides*-related organisms can also effectively dechlorinate PCB congeners.

Antifungal susceptibility testing based on the bioluminescence by *Armillaria cepistipes*, formely unknown to produce light

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The phenomenon of bioluminescence of fireflies, bacteria, and dinoflagellates is known for a long time. Already in ancient times philosophers and scientists studied the light production and emission by living organisms. However, very little research has been carried out on fungal bioluminescence. Today, more than 70 higher fungal species are known to be bioluminescent and new light emitting species are discovered continuously. Luminescent fungi can have a great practical value using them as eukaryotic biosensors for the detection of pollutants and antifungal agents. At this time, all known bioluminescent fungi are Basidiomycetes belonging to four distinct lineages. However, data are available only for a few well known species, including *Armillaria mellea*, *Mycena citricolor*, *Mycena chlorophos*, *Omphalotus olearius* and *Panellus stipticus*. Luminescence may be present in various parts of the fungi. In many bioluminescent *Mycena* and *Armillaria* species, only the mycelium emits light. In others both the mycelium and the basidioms are luminescent, as for example in *P. stipticus* and *O. olearius*. To study the effect of several antifungal agents, we developed a biotest based on the light production of *A. cepistipes*, which was - until today - not known be a light-emitting fungal species. As example, mycelium was exposed to beta-Thujaplicin at concentrations of 0.1, 1, 10, and 100 mg/l and the subsequent alteration of the light emission was measured. We compared the results also regarding the influence of the toxin on fungal biomass production. At high concentrations, beta-Thujaplicin negatively affected luminescence as well as biomass formation. Surprisingly, at low toxin concentrations a stimulation of light emission and biomass production was detectable suggesting hormetic effects of beta-Thujaplicin. In summary, this bioassay provides a rapid and easy practicable method to test possible antifungal substances. It might be particularly useful to find new agents that inhibit the *in vivo* growth of *Armillaria*, because several *Armillaria* species are known to be severe forest pathogens.

Abundance and genotype diversity of *Metarhizium* spp. in a grassland in northern Switzerland

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Metarhizium belongs to the most common insect pathogenic fungal genera worldwide and a variety of *Metharizium* spp. based biological control agents have been developed to control various pest insects. However, knowledge of natural distribution and habitat association of *Metarhizium* is incomplete and only few studies have addressed this topic. Furthermore, a new species concept for the genus *Metarhizium*, based on a multigene phylogenetic approach, has been introduced in 2009 by Bischoff et al., and therefore results of previous studies are difficult to compare.

In this project, diversity of *Metarhizium* spp. in a grassland field in northern Switzerland was studied with different molecular methods, with the aim to compare abundance, species and within species diversity between a permanent and an adjacent 1-year old grassland plot.

120 soil cores were sampled along 4 transects of 100 m per field type. Abundance of *Metarhizium* spp. was determined byplating soil suspensions on a selective medium and counting colony forming units. A total of 120 isolates (60 from each field type) were selected for further analysis. Genotype diversity and distribution in the field were assessed using a set of 18 microsatellite markers whereas species diversity was assessed by sequence analysis of the 5'end of elongation factor 1-α.

First results obtained from the plating method indicated that overall abundance of *Metarhizium* spp. is lower in permanent grassland as compared to the recently established grassland. Moreover, two colony morphology types were consistently isolated across all soil samples. Currently, genotyping and sequencing of the selected isolates are underway. Comparison of genotype occurrence and abundance between the permanent and adjacent 1-year old grassland field as well as correlation of genotypes or species with the morphology types observed will be analyzed.

Are microsatellite analysis and elongation factor 1α sequencing useful tools for discriminating exotic from native *Metarhizium* strains?

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Species of the *Metharizium anisopliae* species complex belong to the best studied fungal pathogens of insects. Recently, the phylogeny of the *M. anisopliae* complex was redefined based on a multigene phylogenetic approach (Bischoff et al., 2009). In this study nine terminal taxa were recognized as species within the *M. anisopliae* species complex and reclassified to *M. majus*, *M. guizhouense*, *M. pinghaense*, *M. anisopliae*, *M. robertsii*, *M. brunneum* and the more distantly relates species *M. lepidiotae*, *M. acridum*, and *M. globosum*. *Metarhizium* spp. infect a broad range of insects including important crop pests such as grasshoppers, caterpillars, beetles, aphids and leafhoppers and they have a great potential as biological control agents (BCAs). Genetic characterization and identification of BCAs as well as knowledge on their natural distribution in the environment is crucial for risk assessment, registration, and monitoring of such products. The aim of this study was to determine whether microsatellite analysis and elongation factor 1α (EF1α)sequencing qualify as a tool for distinction of foreign and Swiss native strains of *Metarhizium* spp.

Analyses were performed on three Swiss *Metarhizium* collections (35, 36, 33 strains), a collection of *Metarhizium* strains from Denmark (15 strains) and a reference collection (46 strains) representing the nine *Metarhizium* species collected worldwide. EF1α sequencing revealed the presence of three species (*M. brunneum*, *M. robertsii*, and *M. guizhouense*)in the Swiss collections and four species (*M. brunneum*, *M. robertsii*, *M. guizhouense*, and *M. majus*) in the Danish collection. Eighteen microsatellite markers were applied for genotyping the strains and assessment of within species genotype diversity. Data will be used to test whether the applied tools allow discrimination of foreign and Swiss strains.

Is the entomopathogenic fungus *Beauveria brongniartii* also an endophyte?

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The ascomycete fungus *Beauveria brongniartii* is a naturally occurring soil fungus and the most important pathogen of the European cockchafer (*Melolontha melolontha*), a pest in permanent grasslands and orchards. Since 1991, *B. brongniartii* has been available as a commercial biological control agent (BCA) in form of fungus colonized barley kernels to control the soil-dwelling larvae of *M. melolontha*. Recently studies have shown that the insect pathogenic fungus *B. bassiana*, a close relative of *B. brongniartii*, is able to grow as an endophyte. This finding raises the question, whether *B. brongniartii* is also able to grow endophytically within plants. If so this might have relevant implications on biosafety aspects of this BCA in agriculture because plants of treated fields serve as animal feed. The goal of this project was to test whether *B. brongniartii* can be isolated from plants in a grassland field treated with the *B. brongniartii* BCA.

9 different plant species were sampled from a field treated with *B. brongniartii* (strain 996) in spring 2012. For each plant species eight replicates were collected (cut 1 cm above ground) along two transects of 50 m. In the laboratory two 1 cm pieces were cut from the lowest part of the stem of each plant sample. Both plant pieces (one was surface sterilized) were incubated on B. *brongniartii* selective medium. After 3 weeks of incubation out-growth of *B. brongniartii* was observed in 4 samples (1 surface sterilized, 3 not sterilized) of the plant species *Trisetum flavescens* and *Anthoxanthum odoratum*. *B. brongniartii* isolates have been obtained from the samples and are now submitted to microsatellite analysis to test whether they represent the applied strain or native *B. brongniartii* strains. Results indicated that *B. brongniartii* has a potential for endophytic growth. However, the fungus was isolated from above ground plant parts at a low frequency only. In a next step strains of *B. brongniartii* will be labeled with green fluorescent protein to investigate the endophytic growth *in situ* in root and stem tissue of different plant species.

Bacterial Spores Involved in Mineral Oxidation and Precipitation

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It has been shown that bacteria can be involved in oxidation or reduction of several minerals. As well, it has also been shown that spores are able to catalyze the reduction of U(VI) to UO_2. The aim of the present study is to investigate whether spores of aerobic endospore forming bacteria are involved in (bio-)chemical reactions leading to mineral formation in environments with high concentration of copper, manganese, magnesium and calcium.

Twelve strains from a collection of 120 endospore-forming bacteria were chosen to be tested for mineral formation in different media containing high concentrations of copper, manganese and calcium. These strains include 6 thermophiles and 6 mesophiles isolated from hot and warm geothermal sites, and have been phenotypically characterized according to their 16S rRNA gene sequences as closely related to *Bacillus thurigiensis* (3), *Anoxybacillus rupiensis*, *Aeribacillus pallidus*, *Bacillus alveayuensis*, *Geobacillus kaustophilus* (2), *Bacillus mycoides*, *Bacillus cereus* (2) and *Bacillus licheniformis*. These strains have also been tested for their biochemical properties for a series of reactions (catalase, oxydase, nitrate reduction, VP, mannitol, citrate, esculine, urea, among others).

After inoculation in media that induce sporulation, bacterial spores have been harvested and inoculated with cooper, manganese and calcium solutions, for several weeks. Change in color and turbidity, as well as mineral precipitation has been observed. Moreover, contrast-phase microscopy as well as scanning electron microscopy has revealed information about the interactions between bacterial spores and their environment.

The observations of biomineralisation are of critical importance when it comes to decontamination of waters and soils from metals such as copper, but also could be adding knowledge to geology concerning the formation of certain minerals. On-going whole genome sequencing of these 12 strains will reveal more information about the molecular basis of the biomineralisation process.

Biological ice nucleation at tropospheric cloud heights

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Biological ice nucleators (IN) are the most abundant agents to catalyse ice formation at warm temperatures ($\geq$ -10 °C). Yet, the relevance of biological ice nucleation for cloud processes, such as initiating precipitation, remains ambiguous. Moreover, very little is known about abundance and nucleation spectra of these IN at tropospheric cloud altitudes. Equally unknown is the relative importance of different kinds of biological IN in this part of the atmosphere, their likely change with seasons, with weather and air mass origin.

The purpose of this project is then to understand meteorological conditions and environmental factors associated to the presence of biological ice nuclei in precipitation. Rain and snow samples are collected at the High Alpine Research Station of Jungfraujoch in the Swiss Alps, 3580 m above sea level, as representative of tropospheric cloud heights. Concentration of IN is determined by a cooling bath apparatus with an innovative system of automatic recording of freezing events. Particular attention is dedicated to the analysis of the role played by bacteria in conditioning this warm temperature nucleation activity. In case of a significant microbial presence in the samples, further isolation of *Pseudomonas syringae*, known as efficient ice nucleator, will be carried out.

Microbial community structures and biogeochemistry of pillow-like sediment structures in Lake Geneva

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Conspicuous pillow-like sediment structures cover large areas of the ground of Lake Geneva. While reasons and mechanisms leading to their formation are still controversial, they represent an excellent opportunity to assess the small-scale heterogeneity of biogeochemical processes and the associated microbial community structures. For this we investigated the geochemistry and microbiology in sediment cores sampled on top of pillow structures and in sediment collected in the trenches between the pillows. The porewater chemistry was determined using whole core squeezing and microsensors, solid phases were characterized by ATR-FTIR. Clone libraries of 16S rRNA genes and organic biomarkers were used to assess the microbial community structure. Microsensor profiles of dissolved oxygen show that sediments are anoxic below a depth of 1-2 mm. Oxygen penetration depth was typically lower and O_2 fluxes were higher in the sediments sampled on top of the pillow structures. This is consistent with porewater concentration profiles of NO_3^-, Fe^{2+}, Mn^{2+}, and SO_4^{2-} indicating more reduced conditions in the pillow sediments than in the trenches between pillows. Despite significant differences in fluxes of solutes, however, pillow and trench sediments show qualitatively similar concentration profiles likely reflecting identical underlying biogeochemical processes.
16S rRNA gene clone libraries of Archaea and Bacteria were constructed to determine the dominant members of the microbial community structure. In all three depth sections of both, pillow and trench sediment cores, the archaeal community was essentially composed of methanogenic microorganisms belonging to several genera of unclassified Methanomicrobiales as well as Methanosarcinales (mainly *Methanosaeta*). The bacterial community was much more diverse and the sampling depth of the clone library was not sufficient to draw conclusions at high phylogenetic resolution. We were able to identify the presence of the more abundant representatives, but could not resolve with certainty the less abundant community members. *Rhodocycladales*, *Spingobacteriales*, *Burkholderiales* and *Xanthomonadales* were the most frequently retrieved bacterial orders in both sediment types. *Desulfobacterales* were mainly found in the pillow sediment and *Anaerolineales* in the trenches. High resolution sequencing will be required to determine unambiguously as to what degree the microbial communities differ in such sediments with similar underlying biogeochemical processes.

Elimination of antibiotic resistance genes by an ultrafiltration pilot plant at the Waste Water Treatment Plant Lausanne, Switzerland

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Antibiotics resistance is increasingly seen as an emerging contaminant in aquatic ecosystems. Reduction of the amount of resistant bacteria in treated wastewater would be one strategy to reduce the dissemination of resistance factors into the environment. A pilot plant combining charcoal activated powder adsorption (CAP) and ultrafiltration (UF) was tested to determine whether the CAP-UF treatment would be effective to eliminate antibiotic-resistant bacteria and associated resistance genes. Samples were taken at the WWTP's sewage inflow (*STEP in*), the outflow of the biological treatment stage and inflow for the pilot (*Bio out*), and from the CAP-UF treated water (UF). Total cell numbers were quantified using flow cytometry. Viable counts of bacteria resistant against three different combinations of antibiotics were counted on R2A agar plates. The abundance of three antibiotic resistance genes (*sul1*, *tetW* and *qnrA*) and bacterial 16S ribosomal RNA gene was determined by real-time quantitative PCR.

The sulfonamide resistance gen *sul1* was present at the highest concentration in the sewage (*STEP in*), followed by tetracycline resistance gene *tetW* with about 50% lower concentration, while the plasmid-mediated quinolone resistance gene *qnrA* was about four orders of magnitude less abundant than the former two genes. The *sul1* and *tetW* genes were detected and quantifiable in all *STEP in* and *Bio out* samples. *qnrA* was only detected in *STEP in*. In UF samples both *sul1* and *tetW* were above the limit of detection only in some samples. The relative abundance of the *sul1* and *tetW* genes in *Bio out* was low, indicating a higher effectiveness for reducing resistant bacteria in water treated with biological nitrogen elimination (*Bio out*) compared to single-stage biological treatment. Overall, our qPCR data confirms that bacteria, resistant bacteria, and resistance genes are effectively eliminated by the UF pilot, often to below the limit of detection, but indicate some regrowth of bacteria, including carriers of resistance genes, after membrane filtration. Advanced wastewater treatment schemes like CAP-UF appear suitable to reduce the release of carriers of antibiotic resistance genes into the environment.

Microsynth's 16S Metagenomic analysis and Transcriptomic analysis

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Microsynth AG presents two of their sequencing and analysis pipelines which supports scientists in their research.

16S Metagenomics - Prokaryotic community analysis

Next-generation sequencing technologies allow the rapid and comprehensive profiling of microbial communities by sequencing parts of the 16S ribosomal DNA (rDNA). However, the library generation, sequencing, and evaluation of sequence data is a challenge due to (i) bias in the amplification step depending on the used primers, (ii) the large amount of data generated, making a manual handling of the data impossible and (iii) technology-specific sequencing patterns that must be addressed properly during the down-stream analysis. Microsynth offers a service which includes all steps (amplification, library preparation, sequencing, data analysis) and provides a high-quality analysis of the sample, while not ignoring some inherent difficulties in the method.

Transcriptomic analysis - Differential expression

mRNA sequencing offers a state-of-the-art approach to generate transcriptional profiles and analyse the transcriptome for differentially expressed (up- or downregulated) genes. Usually RNA is extracted from organisms which persist in two (or more) conditions (e.g. normal vs stressed, wildtype vs mutant, etc.). The mRNA of the sampled cells is sequenced using high throughput sequencing technology; the sequenced RNAs are then assigned (mapped) to the organism‘s genes. Genes which are differentially expressed (up- or downregulated) will have a signficiantly different amount of reads which map to this gene depending on the condition the sample stems from Microsynth offers a service which includes high-throughput sequencing of the samples and a high-quality analysis of the results

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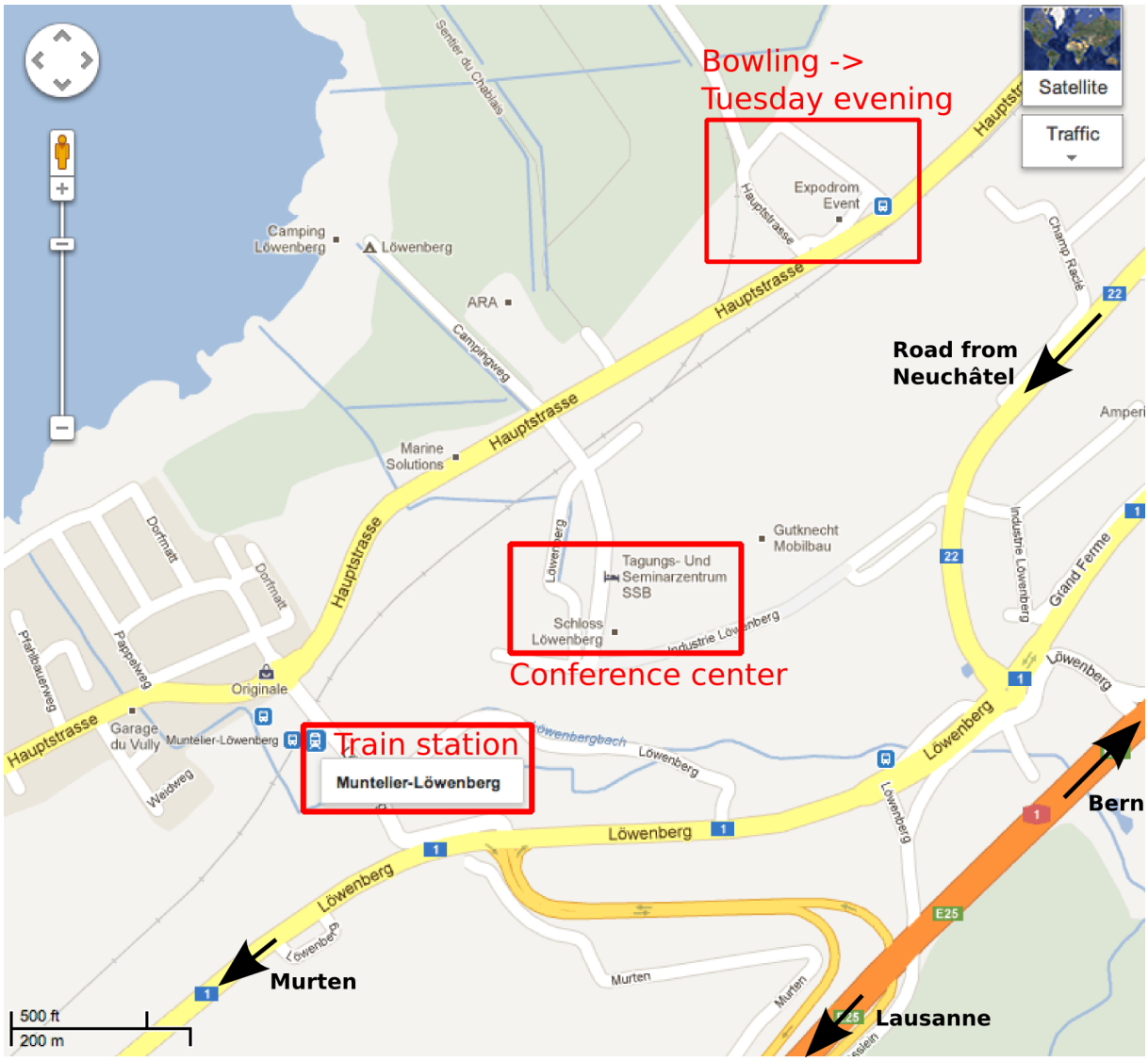
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